DEVELOPMENT OF MICROPROPAGATION PROTOCOL FOR JACKFRUIT (*Artocarpus heterophyllus* Lam.)

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Development of micropropagation protocol for jackfruit (*Artocarpus heterophyllus* Lam.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Place: Padannakkad
Date: 28/11/2020

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CERTIFICATE

Certified that this thesis, entitled **"Development of micropropagation protocol for jackfruit (*Artocarpus heterophyllus* Lam.)"** is a record of research work done independently by Ms. Karishma N.A. (2018-12-021) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

et al.	Co- workers/ Co-authors
MS	Murashige and Skoog
WPM	Woody Plant Medium
NAA	1-Naphthalene acetic acid
IBA	Indole-3-butyric acid
i.e.	That is
Kg	Kilo gram
c.m	Centimetre
MHa	Million hactare
GOI	Government of India
Ha	Hectare
DES GOK	Department of Economics and Statistics, Government of Kerala
g/L	Gram per litre
$HgCl_2$	Mercuric chloride
%	Percent
ml	Milli litre
NaOCl	Sodium hypchlorite Clorox
PVP	Polyvinylpyrrolidone
mg	Milli gram
mm	Milli metre
BA	6- Benzyladenine
µM	Micro molar
mg/L	Milli gram per litre
hrs/day	Hours per day
°C	Degree celsius
IAA	Indole-3-acetic acid
Kn	Kinetin

GA_3	Gibberellic acid
viz.	Namely
cv.	Cultivar
AC	Activated charcoal
RARS	Regional Agricultural Research Station
Dt.	District
TSS	Total soluble solids
^{o}B	Degree brix
HCl	Hydrochloric acid
NaOH	Sodium hydroxide

Introduction

1. INTRODUCTION

An expeditious growth in population coupled with resource scarcity causes malnutrition in many developing countries. Hence, supply of food and adequate nutrition has a greater significance in these countries. So the quality and quantity of food have become great issue at the global level. We can overcome this problem to a certain limit by focusing on the underutilized crops like jack. Due to the limited development of downstream product jackfruit remained as minor fruit in the domestic market. The use of this nutritionally rich food we can meet the nutritional deficiency in developing countries as well as cultivation of this crop provides a sustainable environment through better soil management.

Jack fruit (*Artocarpus heterophyllus* Lam.) is a large sized tree bearing largest fruit belonging to the family Moraceae. It is popular in several tropical and subtropical countries. Ripe fruits contribute to the nutrition as a source of carbohydrates, minerals and carotene, a precursor of vitamin A. In eastern and southern parts of India, tender fruit is popular as vegetable.

Pulp can be used for the preparation of pickles, chips, papad *etc*. Jacalin is a protein extracted from the fruit rind. Jack grows well in humid and warm climate of hill slopes and in arid plains of south India, making it suitable for dry land horticulture. The crop commonly found in southern states like Kerala, Tamil Nadu, Karnataka, and Andhra Pradesh. Jack often referred to as miracle fruit because it has the ability to withstand heat and it can also replace wheat and other staple food under the threat of climate change. The tree mainly valued for its fruit and timber (Shyamalamma *et al.,* 2008).

Jack contains certain bioactive compounds which play an important role in preventing certain diseases (Galaverna *et al*., 2008). The jackfruit seed contains 4.27% ash, 6.73% protein, 73.34% starch, 0.80% fat and 1.6% fiber (Goswani *et al*., 2010). Jack is rich in potassium with 303 mg per 100 g (Swami *et al.*, 2012). The crop is good source of pectin, which makes it ideal for preparation of processed products. There are several factors responsible for low commercial utilization of jack including limited availability of improved varieties, long gestation period for bearing

the fruits, difficulties in harvesting and less acceptance among the people.

Jack is mainly propagated through seeds, but the crop being cross pollinated and highly heterozygous in nature, it shows variation for yield, size, shape, flesh colour, quality of fruit etc. and also seedling takes longer period to bear fruits. Propagation through grafting is highly desirable while number of plants produced through this method is less (Dutton, 1976; Samaddar, 1990). Hence, another method is required which is expected to solve above problems. Micropropagation can be used as an alternative to overcome the limitations of conventional methods because it allow the mass multiplication of uniform planting material within a short period of time under controlled condition.

MS (Murashige and Skoog) medium is commonly used for the micropropagation in jack. WPM (Woody Plant Medium) and B_5 medium are also used for the *in vitro* propagation of this woody perennial plant. MS medium contains almost all the nutrients including minerals, vitamins, growth regulators required for the explant establishment and shoot proliferation. MS medium provided with cytokinin in certain concentration will give good shoot induction and proliferation. Half strength MS medium with auxin (NAA and IBA) alone and in combination gives better rooting in jack (El-Zaher, 2008).

Through *in vitro* propagation large amount of uniform, true to type, high quality, disease free plants can be produced irrespective of the season and weather (Litz *et al.*, 1985; James, 1988; Martin *et al*., 2006).

Therefore, the present study entitled "Development of micropropagation protocol for jackfruit (*Artocarpus heterophyllus* Lam.)" was undertaken with the following objective

- To establish a micropropagation protocol for jackfruit (*Artocarpus heterophyllus* Lam.) using shoot tip explants.

Review of Literature

2. REVIEW OF LITERATURE

Artocarpus heterophyllus Lam., commonly known as jackfruit is a tropical climacteric fruit belonging to mulberry family Moraceae. It is native to Western Ghats of India and commonly found in Asia, Africa, and some regions in South America. It is a rich source of carbohydrates, proteins, vitamins, minerals, fibre, calcium, phosphorus, potassium, magnesium and phytochemicals (Prem *et al.,* 2015). Jack is also referred as poor man's fruit because of its contribution to the food supply when there was an adequate shortage of food grains (Singh *et al.,* 1963).

It is commonly found in warm and moist regions. It is cross pollinated and monoecious, producing male and female inflorescence on the same tree (Bose, 1985). Fruits are produced on the tree trunk *i.e.* cauliflorous in nature. It is an evergreen medium sized, latex producing tree grow up to a height of 8-25 m with a somatic chromosome number of 2n = 4x = 56 (Prakash *et al*., 2009). It is designated as national fruit of Bangladesh and Indonesia.

The jackfruit tree produces the world's largest known edible fruit with an individual fruit weight upto 50 Kg measuring upto 90 cm in length and 50 cm in diameter and yields 20 to 250 fruits per tree per annum, sometimes reaching 500 fruits on a fully mature tree (Haq, 2006; Shyamalamma *et al*., 2008). It has a straight rough stem and a green or black bark which has a thickness of around 1.25 cm, exuding milky latex. Tree is well known for its fruit because of its taste, sweetness, colour, texture, crispness, aroma along with unseasonal fruit bearing capacity and is valued mainly for its timber, fuel, fodder, medicinal and industrial products (Hasan *et al*., 2008).

In India the crop is grown in an area of 185 MHa, which accounts a production of 1830 MT (GOI, 2018). West Bengal, Kerala, Tripura, Karnataka, Tamil Nadu, Goa, Andhra Pradesh, Odisha, Bihar, Uttar Pradesh and Assam are the major states growing jack fruit in India.

In Kerala, 91982 Ha area is under the cultivation of jack with an annual production of 281 million fruits (DES GOK, 2016) and fruit is designated as official fruit of the state. Even though widely grown in the country, it is not considered as a commercial

fruit crop. Long gestation period, limited choice of suitable varieties, availability of limited planting material and variability in yield and quality are some of the constraints in the cultivation of jackfruit (Nunjundaswamy and Mahadevi, 1993). Introduction of high yielding jackfruit varieties adhering to a proper harvesting and post harvesting practices can increase the utilization of jackfruit (Ranasinghe *et al*., 2019).

The most common propagation method of jackfruit is by seed, but the crop being cross pollinated and highly heterozygous, the plants raised from seeds are not true to type. Furthermore, due to the recalcitrant nature of the seeds, its storage even for a short time results in loss of viability and poor germination. The vegetative propagation methods are generally tiresome, time consuming and seasonal with low multiplication rate making it difficult for effective and commercial level propagation. Hence micropropagation can be used to overcome the limitations of conventional vegetative propagation.

Micropropagation can provide a method for the rapid mass multiplication of elite and uniform plantlets within a short period of time from a single explant under controlled environmental condition. This technique not only offer rapid mass multiplication of stock plants but also for the conservation of rare plant species. This approach is important for the production plants which are resistant to biotic and abiotic stresses. Moreover, micro propagation can provide plantlets throughout the year irrespective of seasonal variations and also this technology is being widely used for plant improvement and for the production of secondary metabolites.

According to Beversdorf (1990), micropropagation is an *in vitro* regeneration of plants from protoplast, cells, tissues and organ. Debergh and Read (1991) stated that it is the true-to-type propagation of a selected genotype using *in vitro* culture techniques.

In this chapter, literature on effect of surface sterilization, source of explant, effect of media on initiation, multiplication and rooting of jack, control of browning and phenolic exudation are reviewed.

2.1 Effect of surface sterilization

Controlling fungal and bacterial contamination is difficult in woody plants

explants taken from field source. Hence, surface sterilization plays an important role in minimizing this with minimal damage to plant cells.

A study conducted by Amany *et al*. (2007) on *in vitro* propagation of jack fruit revealed that surface sterilization of jack explant with 3g/L of mercuric chloride ($HgCl_2$) for 1 minute and rinsing with sterilized distilled water three times followed by with 40% Clorox solution containing one drop of Tween 20 per 100 ml of solution as surfactant for 20 minutes is found to be effective for good explant survival.

Zamir *et al.* (2007) opined that, shoot tip collected from the selected plants of guava sterilized with 0.05% mercuric chloride containing Tween 80 as surfactant agitated for 5 minutes with a rotary shaker then immediately washed with sterile distilled water for 3-4 times under laminar air flow cabinet, gave good survival rate.

El-Zaher (2008) conducted a work on micropropagation in jackfruit by using apical growth without leaves and nodal segments as explants. These explants were thoroughly washed under tap water followed by soap solution for 3 minutes and with sterile distilled water for 5 minutes. Explants were soaked in antioxidant solution like ascorbic acid 150 mg/l for 2 hours. These explants were soaked in surface sterilizants like ethanol at 70% for 2 and 5 minutes, $HgCl_2$ at 0.1 for 10 minutes and 0.2% for 5 minutes and Chlorox at 10-20% for 10- 15 minutes. The results have shown that a combination of 70% ethanol for 2 minutes with 0.2% $HgCl_2$ for 5 minutes and 15% Clorox for 15 minutes is the best treatment in terms of good percentage of the survival and aseptic explants.

Singh *et al*. (2010) claimed that sterilization of shoot tip and nodal bud of pomegranate using sodium hypchlorite Clorox (NaOCl) for 20 minutes gave good explant survival.

Liu and yang (2012) carried out a work on clonal propagation of guava *(Psidium guajava).* They used apical shoot having a length of 5-7 cm as explant and washed with running tap water and sterilized with different sterilizants like 15% bleach for 20 minutes, PVP solution with 15% bleach and 15% bleach with PVP in the media. After sterilization treatment explants were washed with sterile water for 5-6 times. Lowest browning rate and maximum explant survival was noticed on last treatment *i.e*18.3 and 53.3 respectively. They reported that 15% bleach with PVP in the media was

the best surface sterilizant for guava explant.

In vitro propagation of mulberry, using 70% ethanol then with 20% Clorox (sodium hypochlorite 5.25%) for dipping duration of 1 and 10 minutes gave the highest survival percentage and lower contamination percentage (Attia *et al*., 2014).

In pomegranate, Kalalbandi *et al*. (2014) reported that maximum survival (90.58%) and minimum microbial contamination (9.52%) was obtained by surface sterilization with 0.1% mercuric chloride for 10 minutes.

Harb *et al*. (2015) used shoot tip segment as explant from 40-50 year old jackfruit tree. They washed the explants under running tap water for 60 minutes for removing dirt and dust adhered on it. The explants were surface sterilized with 70% alcohol and washed with sterilised distilled water 3-4 times in a laminar air flow cabinet. Then immersed in 2.5% sodium hypochlorite (NaOCl) with two drops of Tween 20 for 25 minutes. These surface sterilized explants were washed with sterile distilled water to remove the remaining traces of disinfectant showed better survival rate.

Gayathri and Sathyanarayana (2015) observed that washing of shoot tip and nodal segments of jack with running water and with double distilled water 3-4 times followed by surface sterilization with a combination of bavistin (1000 mg), cetrimide (500 mg) and streptomycin sulphate (100 mg) per liter of water for 90 minutes by giving a continuous stirring then by surface sterilization with mercuric chloride ($HgCl_2$ 0.1%) for 10 minutes gave best result by reducing the infection.

Ali *et al*. (2016) conducted a work on *in vitro* shoot multiplication of jackfruit, observed that most effective sterilising procedure was 0.3% Cocide an antifungal agent for 45 minutes and 70% ethanol for 2 minutes followed by washing with distilled water then by 0.5% $HgCl_2$ for 5 minutes.

2.2 Explants

According to Pierik (1987), some factors affect the growth of explant *in vitro* which includes genotype, plant age, physiological state such as vegetative or regenerative stage, age and stage of explants, position of explant within the plant, general health of explant, size of the explant and method of inoculation etc. So these factors are to be considered while selecting the explant for micropropagation.

2.2.1 Explants for micropropagation

According to Rajmohan and Mohanakumaran (1988) maximum response (17.4 shoots) was obtained from seedling explants followed by stem sprouts from five year old trees *i.e.* 4.5 shoots. Explants excised from ten to thirty year old tree showed a drastic reduction in response *i.e*. 2.80 and 2.09 shoots respectively.

In Ber, maximum number of shoots (15-20) was obtained from shoot tip followed by coteledonary node (10-15), nodal section (8-10) and hypocotyl (6-8) (Mathur *et al*., 1993).

Amany *et al.* (2007) reported highest survival percentage and growth rate in shoot tips of 0.5-1 cm long explants of jack compared to the intermediate and basal nodal cuttings with two nodes.

El-Zaher (2008) reported highest percentage of establishment in apical growth without leaves having a length of 5-10 mm (16.66 per cent) followed by nodal segments having 10-15 mm length (14.42 per cent) and least establishment was obtained with coteledon segment (0 per cent).

According to Azam and Rahmatullah (2009) highest survival rate was obtained with 10-15 day old newly sprouted shoot tips from 10-12 year old jack tree.

Shoot tip explants of 5 cm long collected from jack in the March and cultured on MS medium resulted in highest survival and bud induction (Ashrafuzzaman *et al.*, 2012).

Nikumbhe *et al.* (2013) tested influence of different types of explants of pineapple such as crown apical section, slips apical section and shoot tip of suckers on establishment of aseptic culture. They found that slips of apical section had highest aseptic culture (78.13 per cent) followed by crowns apical section (69.64 per cent), and shoot tip of suckers (52.59 per cent).

According to Pant and Kabade (2014) maximum bud induction in Lakoocha were obtained within 7.33 days by using nodal explant with axillary bud as explant.

Harb *et al*. (2015) studied the influence of seasonal variation in bud break in *in vitro* propagation of jack fruit. They reported maximum survival per cent (100) in shoot tips and nodal segments collected in winter with minimum browning compared to those collected in spring, summer, and autumn.

Gayathri and Sathyanarayana (2015) used shoot tip and nodal segments as explants. They reported highest percentage of shoot establishment of 75 per cent with 8 per cent fungal contamination and 17 per cent bacterial contamination with shoot tip collected in January, and also nodal segments collected in the same month had shown highest survival of 50 per cent with 20 and 30 per cent fungal and bacterial contamination respectively.

Shatnawi *et al.* (2019), reported shoot tip explants of 3-5 mm in length from 10 year old trees of fig gave better bud induction than nodal segments.

2.3 Effect of media growth regulators on establishment, multiplication and rooting

Roy *et al*. (1993) described a method for propagation of flood tolerant jack fruit by *in vitro* culture. Better shoot bud formation was obtained with a combination of BA and NAA in MS medium which was 8.88 µM and 2.68 µM respectively. Eighty per cent rooting occurred on half strength MS salts with 5.37 µM NAA and 4.92 µM IBA.

Roy and Roy (1996) successfully induced shoot and root organogenesis from shoot bud explant of jack. The shoot bud induction was maximum on MS medium added with 2.5mg/L BA and 0.5mg/L NAA. They also claimed that lowering the concentration of BA and NAA to 1.25mg/L and 0.25mg/L and addition of 15 per cent coconut milk enhanced the number of shoots to 40. Rooting was achieved with the addition of 1mg/L NAA and IBA to the half strength MS medium.

Lee and Keng (2005) developed a rapid clonal propagation method in jack through *in vitro* shoot proliferation from apical shoot of 12 year old tree. Maximum shoot formation was recorded on MS medium supplemented with 4.5 mg/L BA within three to four weeks of culture. Rooting occurred on half strength MS medium supplemented with a combination of 0.5 mg/L NAA and IBA and provided with a darkness for ten days later exposed to a light for 3 weeks resulted in normal healthy and long roots.

Mannan *et al*. (2006) carried out a study to know the effect of time of the year and growth regulators on *in vitro* propagation of jackfruit. They used shoot tips from fresh sprouts on the trunk of mature jackfruit tree as explant. These were collected in three seasons (September, October, January) and cultured on MS medium with growth

regulators. Maximum survival rate and response to proliferation were obtained from explants collected in January (95.56 per cent) than other two season. Medium fortified with 1 and 1.5mg/L BA showed 100 per cent bud proliferation. Highest rate of multiplication (2.33) and longest shoot (1.45) were achieved by subculturing the shoot tip on MS medium with 0.5mg/L NAA and 1.5mg/L BA.

According to Amany *et al*. (2007) jackfruit explants such as shoot tips, intermediate and basal nodes cultured on MS medium fortified with 3.0 mg/L BA and 0.1 mg NAA produced the highest number of shoots, leaves/explant and shoot length. MS medium was the best medium in term of average number of shoots, as it recorded the highest number of shoots /explant (27.33) followed by B_5 medium (18.58). More number of roots was obtained on half strength MS medium supplemented with 1.50 mg/L IBA. They incubated all the culture jar at 27 $\pm$ 2°C under fluorescent light with intensity of 2000 lux for 16 hrs/day.

Tuia and Taylor (2007) cultured juvenile explants of bread fruit obtained from trimmed root suckers on woody plant medium and MS medium. They found that MS medium with 2.5 mg/L BA produced six buds per explant compared to four buds per explant obtained in woody plant medium. They also found that shoot cultured on WPM achieved 76 per cent root growth with 2.7 cm root length.

Azam and Rahmatullah (2009) studied micropropagation of a year round fruiting variety of jack in Bangladesh. Shoot tip and nodal segments were excised from fruit bearing trees used as explant and were cultured on MS medium supplemented with various growth regulators. They found that medium with 3.5 mg/L BA induced more sprouting and regeneration of shoots. Highest multiplication rate was achieved with shoot tips in the presence of 3.5 mg/L BA and 1.5 mg/L Kinetin. They also observed that presence of 0.1 mg/L indole-3-acetic acid (IAA) and 20 per cent coconut water considerably increased shoot elongation and growth of the shoots. The excised shoot rooted well (80% rooting) on half MS medium with 1.2 mg/L IBA (indole-3-butyric acid).

Khan *et al*. (2010) described a method for micropropagation of jack fruit for shoot and root proliferation by using 2-3 cm long shoot tip and nodal segments as explants. Maximum number of shoots (4.66) was achieved in MS medium fortified with 1.5

mg/L BAP followed by 2.0 mg/L BA (2.66 shoots). Highest per cent (80) of rooting occurred when shootlets were cultured on MS medium with 3.0 mg/L IBA followed by 2.0 mg/L which resulted in 70 per cent rooting.

Shoot bud culture in Lakoocha Roxb was studied by Pant and Kabeda (2014). They claimed that nodal explant with an axillary bud when used as explant showed *in vitro* bud induction within 7.33 days on MS medium fortified with 13.33 BA, 2.28 IAA and GA_3 0.57 µM. The best response of shoot multiplication (6.66 shoots/ explant) was recorded on medium containing 13.33 BA and IAA 1.14 µM. The highest rooting of the shoot was achieved on half strength MS medium supplemented with IBA 9.85, BAP 0.89 µM and activated charcoal 500 mg/L i.e. 88.16 per cent rooting.

In jack, shoot and root organogenesis was studied by Harb *et al.* (2015). They reported maximum multiple shoot of 6.6 produced on MS medium supplemented with 2.0 mg/L BA and 0.5 mg/L Kn. Highest shoot length of 5.14 was obtained on medium containing 3.0 mg/L gibberellic acid (GA_3). Highest rooting percentage (80%), average number of roots/explant (4.3) and average root length (4.0 cm) were noticed on medium containing 1.0 mg/l of both IBA and NAA.

Miro and Acedo (2015) developed a protocol for *in vitro* regeneration of plants via shoot tip and nodal segments from fruiting plants and grafted trees of jack. High survival rate was obtained with shoot tip from grafted plants than the mature trees, at the same time phenolic exudation caused tissue browning. MS medium with 3 mg/L BA, 0.5 mg/L GA_3, 0.1 mg/L IBA resulted 2-3 multiple shoot per explant and better growth than MS alone or with 5 mg/L BAP. They found that browning was the major problem which was not minimized by carbon containing medium as well as with frequent subculturing and shoots did not produce roots.

Various explants *viz*., shoot tip, single nodal segment, double nodal segment, lateral shoot bud and leaf petiole of jamun (*Syzygium cuminii* L.) cv. AJG-85 were used as source of explant and shoot tip was found best for establishment of aseptic culture. Higher number of shoots per explants, length of shoots and number of leaves per shoot were achieved on half strength WPM. Better shoot length, number of leaves and number of adventitious buds per explants were attained on 2 mg/L BAP and 0.1

mg/L NAA. GA_3 at 2 mg/L showed better response in terms of shoot length and number of leaves per shoot. Highest degree of rooting in terms of number of roots, root length and number of roots were obtained on medium with 2 mg/L IBA (Prabhuling *et al.*, 2017).

Micropropagation studies in fig were carried out from shoot tip explants by Shatnawi *et al*. (2019) to develop a protocol for micropropagation of true to type plants. Half strength MS medium with 30 g/L sucrose showed better induction. The incorporation of 0.4 mg/L BAP in combination with 0.2 mg/L IBA favoured maximum multiplication rate of 4.2 shoots per explant. Rooting of the shoots were achieved on same medium fortified with 1.5 mg/L IBA.

2.4 Browning and phenol exudation

Browning of explant and culture medium were the major problem in the establishment of *in vitro* culture due to phenolic substances and secondary metabolites from cut surfaces which blocks the uptake of nutrients. These problems can be overcome by the adopting certain techniques like transfer of explants in fresh medium at short intervals, place the culture in dark, use of activated charcoal (AC), polyvinyl pyrrolidone (PVP), the addition of antioxidants to culture medium etc.

In jackfruit, Rajmohan and Mohanakumaran (1988) reported use of 1 per cent activated charcoal and GA_3 1mg/L in the establishment medium, insoluble PVP in the proliferation medium and frequent subculturing minimized the problem of phenolic interference. MS medium supplemented with of 1 per cent activated charcoal and GA_3 1mg/L recorded highest survival rate and produced healthy cultures.

Kumar & Tiwari (2001) described that use of absorbing agents such as activated charcoal, polyvinylpyrrolidone (PVP) resulted reduction in phenolic exudation.

Antioxidant solutions like citric acid (75 mg/l) and ascorbic acid (50 mg/l) were effective in controlling phenolic exudation in guava (Meghwal *et al*., 2003).

Murkute *et al*. (2004) suggested rapid subculturing control the medium browning in pomegranate.

In apple, release of phenolic compounds from the shoots minimized by use of dark treatment for few days (Dalal, 2006).

Nishchal *et al*. (2018) concluded that among the various antioxidants like citric acid, ascorbic acid, activated charcoal and PVP used ascorbic acid 300mg/L resulted minimum phenolic exudation and maximum shoot regeneration.

Materials and Methods

3. MATERIALS AND METHOD

The present investigation on "Development of micropropagation protocol for jackfruit (*Artocarpus heterophyllus* Lam.)" was carried out in the plant tissue culture laboratory of Regional Agricultural Research Station (RARS, North zone), Pilicode, Kasargod (Dt.) during the year 2018-2020.

3.1 Experimental site

All the three experiments were conducted at RARS, Pilicode, located at $12^0 12^{'}$N latitude and $75^0$10'E longitude and at an altitude of 15m above mean sea level.

3.2 Agroclimatic condition

Regional Agricultural Research Station, Pilicode, Kasaragod, experiences a humid climate and comes under tropical humid region. Research station belongs to NARP Northern zone of the state of Kerala and AZ 109^{th} climatic zone of the country.

3.3 Experimental material

3.3.1 Source of explant

An experiment was conducted by Nimisha (2016) on jackfruit types in Kasaragod district through survey and identification, and had shortlisted certain promising types such as early bearing, seedless, gumless and cluster bearing. Later in 2018, Ajeesh had carried out a field evaluation study on 10 promising jackfruit types in Kasaragod district. The maximum number of fruits per cluster was observed in KJ 182 (cluster type) with 8 fruits/cluster and the fruits matured one after another, which ensured a long harvesting period in clustered jack types, in addition it has high TSS (total soluble solids) content (31.47^oB). So, multiplication of this tree is essential, for this shoot tip of cluster jack is used as explant for rapid multiplication (Plate 1, 2). The tree is located at Chirapuram near Nileshwar, Kasaragod district.

3.4. Effect of different media for the establishment of jackfruit explant

Design of experiment: CRD

Replications: 4

Treatments: 5

T_1: MS medium

T_2: ½ MS medium

T_3: Modified MS medium

T_4: Woody plant medium (WPM)

T_5: Gamborg B_5 medium

2 mg/L BA is added to each treatment. Modified MS medium was prepared by halving the concentration of macro elements while retaining the other concentration same as MS medium.

3.4.1 Basal Media

3.4.1.1 Composition of media and its stock solutions

MS medium, half MS medium, modified MS medium, Woody plant medium, Gamborg B_5 medium were used for the study. Chemical composition of these media are given in annexure 1.

Stock solutions of macro nutrients (stock A), micro nutrients (stock B), various vitamins (stock C), iron EDTA solution (stock D) and growth regulators required for these five media were prepared separately by dissolving specific quantity of each chemical in little quantity of double distilled water and make up to required volume with double distilled water.

3.4.1.2 Preparation of media

Required quantities of stock solutions of major salts, minor salts, vitamins, and iron EDTA solution of the five media were added to each beaker. 0.1 g myoinositol, 30 g sucrose were added to the five beakers and dissolved. 2 mg/L BA was pipetted out in these five treatments. The volume made up to one litre by using double distilled water and for the first three treatments pH of the media was adjusted to 5.69 and for rest of the two treatments, to 5.75, by using 1 N HCl or 1 N NaOH. Each medium was boiled and 6 g agar was added to the boiling medium and stirred well to get a homogenise medium. The hot medium was poured to the culture bottles at the

Plate 1: General view of KJ 182

Plate 2: Shoot tip explant for inoculation

rate of 25-30ml/ bottle. Then bottles were sealed with cap and autoclaved at 15 psi pressure and 121^0C temperature for 20 minutes. After sterilization, the medium were cooled at room temperature in a clean dry area.

3.4.2 Culture condition

Cultures were incubated under fluorescent light (2000 lux) at 25°C ± 3 with a 16-hour photoperiod.

3.4.3 Collection of explants

Shoot tip from current season growth of mature tree of KJ- 182 (Chirapuram cluster) jack located at Chirapuram in Kasargod district was used as explant.

3.4.4 Excision and surface sterilization of explants

Shoot tip along with leaves collected from jack were used as explant. After removing the leaves shoot tips were cut into 5 cm long pieces and dipped in sterilized distilled water along with detergent solution. And kept for 10 minutes. After that, explants were washed with sterilized distilled water for two-three times and then were treated with SAAF (1 g SAAF in 200 ml water) for one hour (Plate 3a). Then the explants were washed with sterilized distilled water for five to six times and the cleaned explants were taken to laminar air flow chamber for further treatment. In laminar air flow chamber, all the explants were surface sterilized with mercuric chloride (0.8 g mercuric chloride in 1000 ml distilled water) (Plate 3b). Duration of the treatment was 10 minutes and they were rinsed with sterile distilled water for five to six times to remove the traces of $HgCl_2$.

3.4.5 Inoculation of explants

The explants were cut into appropriate size (3 cm) aseptically for inoculation. Explants which were surface sterilized and aseptically excised were placed on each medium with the help of sterile forceps under sterilized laminar air flow chamber. 25 explants were inoculated for each treatment. During inoculation of the explants care was taken to keep them in proper position.

3.4.6 Observations

The important biometric observations were the following.

3.4.6.1 Number of days to shoot emergence

Number of days taken by each explant for shoot initiation was counted from the date of inoculation to shoot emergence.

3.4.6.2 Number days for shoot multiplication and branching

Number of days taken to shoot multiplication and branching were counted from the day of inoculation for shoot multiplication and branching.

3.4.6.3 Number of shoot/ explant

Number of shoots developed from each explant were counted separately after four weeks from the day of inoculation.

3.4.6.4 Shoot length (cm)

After four weeks of inoculation the bottle containing shoots were taken out under a laminar air flow chamber and length of shoot was measured from base to tip of the shoot with the help of a sterilized scale. Length of shoot was recorded in centimetre. For every replication 3 shoots were measured.

3.4.6.5 Number of leaves/ shoot

Leaves from three selected shoots from each treatment were recorded separately.

3.5 Shoot multiplication

3.5.1 Effect of different concentrations of growth regulator BA with best medium from the 1st experiment, for shoot multiplication.

Design of experiment: CRD

Replications: 4

Treatments : 5

T_1: Selected basal medium

T_2: Best medium from the 1st experiment

T_3: T_2 + 1 mg/L BA

T_4: T_2 + 2 mg/L BA

T_5: T_2 + 3 mg/L BA

Plate 3a: Explant treated with SAAF

Plate 3b: Explant treated with $HgCl_2$

Results obtained from these treatments as per technical programme were not satisfactory hence, another combination of growth regulators were tried for shoot multiplication. It showed better response in all the growth parameters compared to previous one.

3.5.2 Effect of different concentrations of growth regulator BA and Gibberelic acid (GA_3) with best medium from the 1st experiment, for shoot multiplication.

Different concentrations of BA in combination with 0.35 mg/L GA_3 were used in this experiment. MS medium was used for shoot multiplication.

Design of experiment: CRD

Replications: 3

Treatments : 8

T_1 : MS medium + 1 mg/L BA + 0.35 mg/L GA_3

T_2 : MS medium + 1.5 mg/L BA + 0.35 mg/L GA_3

T_3 : MS medium + 2 mg/L BA + 0.35 mg/L GA_3

T_4 : MS medium + 2.5 mg/L BA + 0.35 mg/L GA_3

T_5 : MS medium + 3 mg/L BA + 0.35 mg/L GA_3

T_6 : MS medium + 3.5 mg/L BA + 0.35 mg/L GA_3

T_7 : MS medium + 4 mg/L BA + 0.35 mg/L GA_3

T_8 : MS medium + 4.5 mg/L BA + 0.35 mg/L GA_3

3.5.3 Subculturing

After 28 days of inoculation the shoots developed were taken out from the culture bottle inside laminar air flow chamber, under aseptic condition. Shoots with excess growth of callus were subcultured after removal of callus and dead region using a sterilized blade and then multiple shoots were separated and transferred to the culture bottles with various treatments. Three shoots were placed in each culture bottle and 25 bottles were used for each treatment. Bottles were placed on rack and subsequent sub culturing was done at 21 days intervals.

3.5.4 Observations recorded

3.5.4.1 Number of days to shoot emergence

Number of days taken by the plantlet for shoot emergence was counted from the day of sub culturing and separately for each treatment.

3.5.4.2 Number of shoot/ explant

Number of multiple shoots were recorded and expressed as number of shoots per explant.

3.5.4.3 Shoot length (cm)

Plantlets were taken from the bottle and cleaned by removing the callus growth and shoot length was measured with the help of a sterilized scale. Shoot length was measured from base of the plant to shoot tip inside a laminar air flow chamber and expressed in centi meter.

3.5.4.4 Number of leaves/ shoot

Leaves were formed recorded separately for each treatment.

3.6 Effect of different concentrations of growth regulators and its combinations on rooting.

3.6.1 NAA and IBA

Design of experiment: CRD

Replications:3

Treatments : 9

T_1: ½ strength of selected basal medium

T_2: T_1 + 1 mg/L NAA

T_3: T_1 + 3 mg/L NAA

T_4: T_1 + 0.5 mg/L IBA

T_5: T_1 + 1 mg/L IBA

T_6: T_1 + 1 mg/L NAA + 0.5 mg/L IBA

T_7: T_1 + 1 mg/L NAA + 1 mg/L IBA

T_8: T_1 + 3 mg/L NAA + 0.5 mg/L IBA

T_9: T_1 + 3 mg/L NAA + 1 mg /L IBA

Apart from the treatment combination as per technical programme another combinations of growth regulator were tried for rooting.

3.6.2 NAA, IBA, BA and GA_3

Design of experiment: CRD

Replications:3

Treatment: 8

T_1: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA

T_2: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA

T_3: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 0.5 mg/L IBA

T_4: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L IBA

T_5: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA + 0.5 mg/L IBA

T_6: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA + 1 mg/L IBA

T_7: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA + 0.5 mg/L IBA

T_8: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA + 1 mg/L IBA

After 7-8 subculturing, the shoots were transferred to rooting medium with various growth regulators alone and its combination. After taking the shoots from culture bottle, callus growth was removed and placed on the medium which containing charcoal (0.45g) and various combinations of growth regulators then culture was maintained for three months for rooting.

3.6.3 Observation

3.6.3.1 Number of days to root emergence

3.6.3.2 Number of roots/ shoot

3.6.3.3 Root length (cm) / shoot

3.6.3.4 Number of secondary roots

3.7 Statistical analysis

The observations recorded were subjected to statistical analysis using WASP 2.0 software prepared by ICAR Research Complex for GOA and OPSTAT.

Results

4. RESULTS

The present study entitled "Development of micropropagation protocol for jackfruit (*Artocarpus heterophyllus* Lam.)" was carried out at RARS Pilicode, Kasaragod during 2018-2020. In this study shoot tip of KJ 182 (cluster type) jack a promising jack type surveyed and identified from Kasaragod by Nimisha (2016) and further evaluated by Ajeesh (2018) was used. The study results are detailed in this chapter.

4.1 Effect of different media for the establishment of jackfruit explant

In this experiment five media were used as treatment which includes MS medium, ½ MS medium, Modified MS medium, Woody plant medium and Gamborge B_5 medium and all these media were supplemented with 2 mg/L BA (Plate 4). This experiment was conducted with an objective to find best basal medium for the establishment. The results of various observations are mentioned below (Table 1).

4.1.1 Number of days to shoot emergence

Number of days to shoot emergence varied from 13.16 to 16.08. Minimum days (13.16) were recorded by T_1 followed by T_3 (13.99), which were found to be on par. T_4 recorded maximum days for shoot emergence (16.08). All other treatments took intermediate number of days for shoot emergence.

4.1.2 Number of days for shoot multiplication and branching

From Table 1 it is evident that, the number of days for shoot multiplication and branching was found significantly different among the media. Among the different treatment, minimum days for shoot multiplication and branching (15.83) was noticed on T_1 which was on par with T_3. Maximum number for shoot multiplication and branching was observed in T_4 (18.66) followed by T_5 (18.58).

4.1.3 Number of shoot/ explant

Data presented in Table 1 indicated that the number of shoot per explant varied significantly among media. Highest number of shoot per explant (1.83) was observed in T_1 followed by T_3 (1.41). The lowest number of shoot per explant (1.00) was found in T_4 followed by T_2 and T_5 (1.16).

Table 1: Effect treatments on culture establishment of jackfruit explant

Sl. no	Treatments	No. of days to shoot emergence	No. of days for shoot multiplication and branching	No. of shoot/ explant	Shoot length (cm)	No. of leaves/shoot
1	T_1	13.16^{c}	15.83^{c}	1.83^{a}	1.13^{a}	1.66^{a}
2	T_2	14.41^{bc}	17.33^{b}	1.16^{b}	0.95^{b}	1.08^{b}
3	T_3	13.99^{c}	16.08^{c}	1.41^{ab}	0.90^{bc}	0.74^{bc}
4	T_4	16.08^{a}	19.66^{a}	1.00^{b}	0.76^{c}	0.33^{c}
5	T_5	15.41^{ab}	19.58^{a}	1.16^{b}	0.82^{bc}	1.00^{b}
SE(±m)		0.41	0.40	0.15	0.05	0.14
C. D. at 5 % level		1.25	1.20	0.47	0.17	0.44

- T_1: MS medium
- T_2: ½ MS medium
- T_3: Modified MS medium
- T_4: Woody plant medium
- T_5: Gamborge B_5 medium

Note : 2 mg/L BA is added to each treatment
Modified MS medium – Concentration of macro elements were made into half.

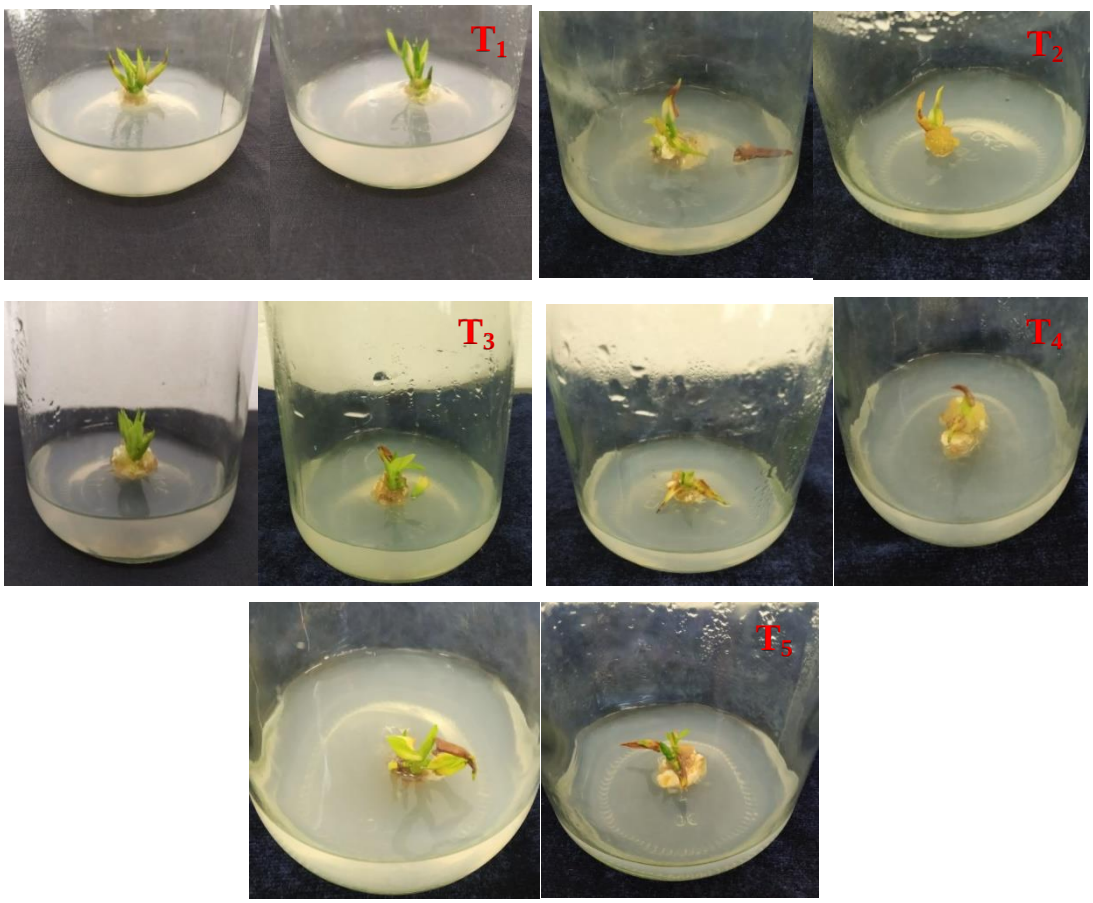


Plate 4: Effect of treatments on culture establishment in jackfruit explant

- T_1: MS medium with 2 mg/L BA
- T_2: ½ MS medium with 2 mg/L BA
- T_3: Modified MS medium with 2 mg/L BA
- T_4: Woody plant medium with 2 mg/L BA
- T_5: Gamborge B_5 medium with 2 mg/L BA

4.1.4 Shoot length (cm)

The mean length of shoot varied from 0.76 to 1.13 cm. Maximum shoot length was observed in T_1 (1.13) followed by T_2 (0.95). The minimum length of shoot was recorded in T_4 (0.76) followed by T_5 (0.82).

4.1.5 Number of leaves/shoot

Data given in this Table 1 regarding number of leaves per shoot revealed that, there was a significant difference among the media with respect to number of leaves per shoot. T_1 (1.66) recorded maximum number of leaves followed by T_2 (1.08) which was on par with T_5 (1.00). Minimum number of leaves recorded in T_4 (0.33) followed by T_3 (0.74).

4.2 Shoot multiplication

4.2.1 Effect of different concentrations of growth regulator BA with best medium from the 1st experiment, for shoot multiplication.

From the first experiment it was clear that among the basal media MS medium with 2mg/L BA was the best with respect to certain characters (it took minimum days for shoot emergence, multiplication and branching, produced highest number of shoot/ explant, shoot length and number of leaves/ explant), hence this medium was selected for multiplication stage.

4.2.1.1 Number of days to shoot emergence

The result regarding the responsiveness of growth regulator to no. of days to shoot emergence presented in Table 2. It is evident from the table that, a minimum of 13 days was taken by T_3 for shoot emergence followed by T_2. While T_1 took more number of days for shoot emergence (16.83) (Plate 5a).

4.2.1.2 Number of shoots/ explant

No. of shoots/ explant ranged from 1.00 to 1.99. Significantly maximum no. of shoots/ explant (1.99) was noticed in T_3. The minimum no. of shoots/ explant was observed in T_1.

Table 2: Effect of treatments on shoot emergence, no. of shoot, shoot length and no. of leaves/ shoot

Sl no.	Treatments	No. of days to shoot emergence	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ shoot
1	T_1	16.83^{a}	1.00^{c}	0.76^{c}	1.08^{b}
2	T_2	13.16^{bc}	1.83^{ab}	1.13^{a}	1.62^{a}
3	T_3	13.00^{c}	1.99^{a}	1.00^{ab}	1.66^{a}
4	T_4	13.33^{bc}	1.66^{ab}	0.90^{bc}	1.33^{ab}
5	T_5	14.33^{b}	1.22^{bc}	0.89^{bc}	1.33^{ab}
SE(±m)		0.41	0.18	0.07	0.13
C. D. at 5 % level		1.26	0.57	0.22	0.41

T_1: Selected basal medium
T_2: Best medium from the 1st experiment
T_3: T_2 + 1 mg/L BA
T_4: T_2 + 2 mg/L BA
T_5: T_2 + 3 mg/L BA

4.2.1.3 Shoot length (cm)

The shoot length varied from 0.76 to 1.13 cm. significantly maximum response to shoot elongation was noticed in the treatment T_2 followed by T_3. The treatments T_5 and T_4 were at par. Significantly minimum response to shoot length was recorded in the treatment T_1.

4.2.1.4 Number of leaves/ shoot

Among the various concentration of growth regulator tried, the treatment T_3 produced maximum leaves(1.66) which was at par with T_2 (1.62). The minimum number of leaves per shoot (1.08) was recorded by T_1.

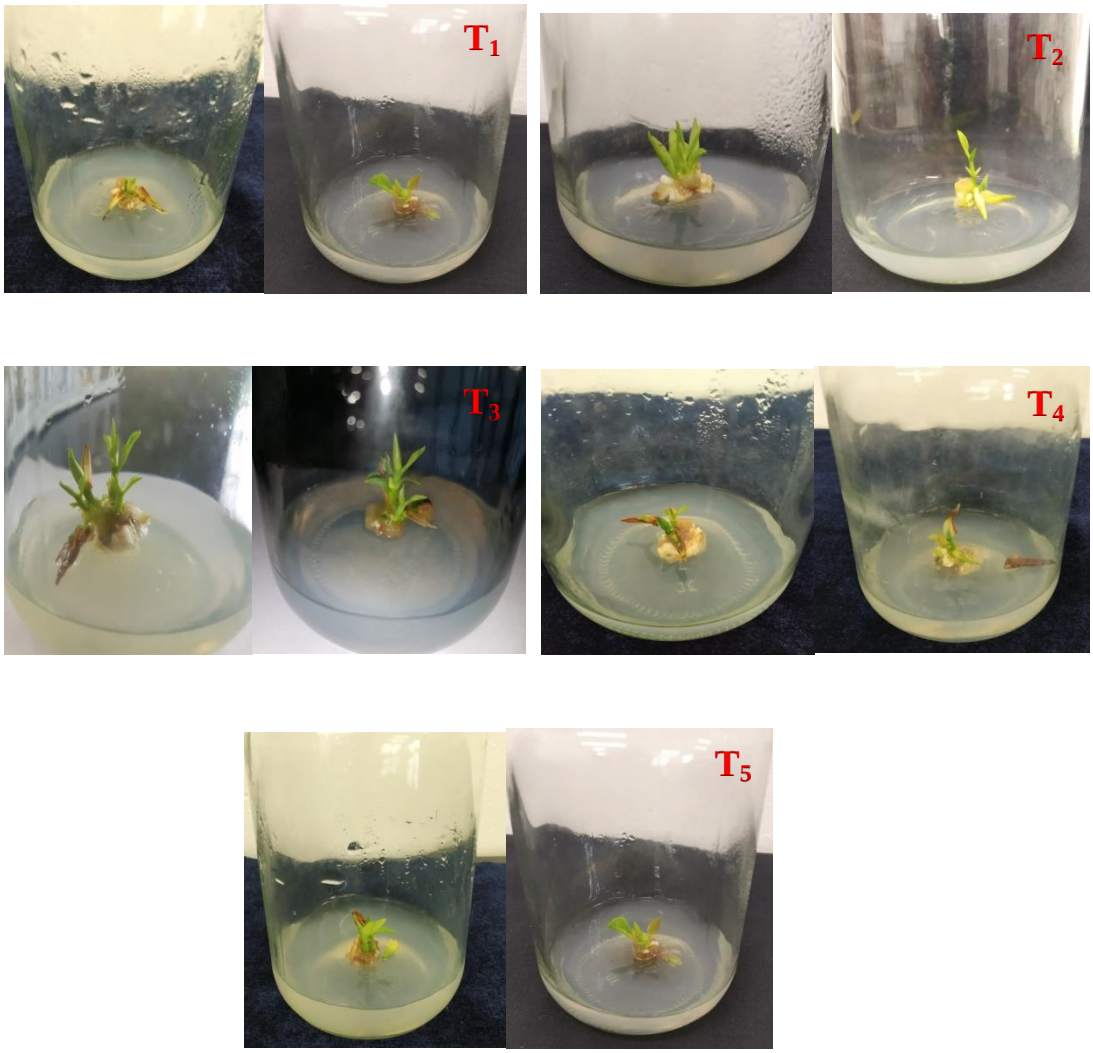


Plate 5a: Effect of treatments on culture establishment in jackfruit explant

T_1: Selected basal medium
T_2: Best medium from the 1st experiment
T_3: T_2 + 1 mg/L BA
T_4: T_2 + 2 mg/L BA
T_5: T_2 + 3 mg/L BA

4.2.2 Effect of different concentrations of growth regulator BA and Gibberelic acid (GA_3) with best medium from the 1st experiment, for shoot multiplication.

Table 3.1: Effect of growth regulators on shoot characters of *in vitro* culture of jack (1st subculture)

Sl no.	Treatments	No. of days to shoot emergence	No. of shoots/ explant	Shoot length (cm)	No. of leaves/ shoot
1	T_1	14.10^{ab}	1.77^{bc}	1.13^{bcd}	1.88^{bc}
2	T_2	14.33^{ab}	1.99^{abc}	1.27^{abcd}	2.10^{abc}
3	T_3	12.10^{bc}	2.03^{abc}	1.31^{abc}	2.55^{ab}
4	T_4	10.22^{c}	2.22^{ab}	1.55^{a}	3.10^{a}
5	T_5	13.55^{ab}	2.38^{ab}	1.08^{bcd}	2.88^{ab}
6	T_6	14.10^{ab}	2.44^{a}	1.47^{ab}	2.77^{ab}
7	T_7	14.55^{ab}	1.55^{cd}	1.00^{cd}	1.44^{c}
8	T_8	14.88^{a}	1.11^{d}	0.90^{d}	1.11^{c}
SE(±m)		0.89	0.21	0.13	0.35
C. D. at 5 % level		2.69	0.63	0.39	1.05

Note :

T_1 : MS medium + 1mg/L BA + 0.35 GA_3

T_2 : MS medium + 1.5mg/L BA + 0.35 GA_3

T_3 : MS medium + 2mg/L BA + 0.35 GA_3

T_4 : MS medium + 2.5mg/L BA + 0.35 GA_3

T_5 : MS medium + 3mg/L BA + 0.35 GA_3

T_6 : MS medium + 3.5mg/L BA + 0.35 GA_3

T_7 : MS medium + 4mg/L BA + 0.35 GA_3

T_8 : MS medium + 4.5mg/L BA + 0.35 GA_3

Table 3.2: Effect of growth regulators on shoot characters of *in vitro* cultureof jack (2nd subculture)

Sl. No.	Treatments	No. of days to shoot emergence	No.of shoot/explant	Shoot length (cm)	No.of leaves/shoot
1	T_1	10.33^{abc}	1.66^{bc}	1.12^{b}	2.33
2	T_2	10.33^{abc}	1.77^{bc}	1.16^{b}	2.55
3	T_3	9.10^{cd}	1.88^{abc}	1.15^{b}	2.67
4	T_4	8.22^{d}	2.11^{ab}	1.69^{a}	3.10
5	T_5	9.66^{bcd}	2.55^{a}	1.08^{b}	2.88
6	T_6	11.10^{ab}	2.10^{ab}	1.23^{b}	2.77
7	T_7	11.88^{a}	1.55^{bc}	1.07^{b}	2.32
8	T_8	12.10^{a}	1.22^{c}	0.90^{b}	1.99
SE(±m)		0.63	0.22	0.14	0.39
C. D. at 5 % level		1.91	0.69	0.42	N/A

Table 3.3: Effect of growth regulators on shoot characters of *in vitro* culture of jack (3rd subculture)

Sl. No.	Treatments	No. of days to shoot emergence	No. of shoot/explant	Shoot length (cm)	No. of leaves/shoot
1	T_1	11.88^{ab}	1.77^{bcd}	1.09^{b}	2.55^{ab}
2	T_2	12.22^{ab}	1.77^{bcd}	1.25^{b}	2.66^{ab}
3	T_3	10.66^{b}	1.88^{bcd}	1.22^{b}	2.66^{ab}
4	T_4	8.51^{c}	2.33^{ab}	1.74^{a}	3.10^{a}
5	T_5	11.44^{ab}	2.66^{a}	1.00^{b}	3.03^{a}
6	T_6	12.09^{ab}	2.20^{abc}	1.33^{ab}	2.99^{a}
7	T_7	12.32^{ab}	1.65^{cd}	1.00^{b}	2.22^{bc}
8	T_8	12.44^{a}	1.44^{d}	0.96^{b}	1.88^{c}
SE(±m)		0.55	0.19	0.14	0.19
C. D. at 5 % level		1.68	0.57	0.44	0.58

Table 3.4: Effect of growth regulators on shoot characters of *in vitro* culture of jack (4th subculture)

Sl. No.	Treatments	No. of days to shoot emergence	No. of shoot/explant	Shoot length (cm)	No. of leaves/shoot
1	T_1	12.10^{bc}	1.77^{bdc}	1.52^{ab}	2.55^{b}
2	T_2	11.88^{bcd}	1.88^{bcd}	1.79^{a}	2.66^{b}
3	T_3	10.88^{de}	1.99^{bcd}	1.52^{ab}	2.77^{b}
4	T_4	10.22^{e}	2.33^{ab}	1.94^{a}	3.44^{a}
5	T_5	11.21^{cde}	2.66^{a}	1.49^{ab}	2.88^{ab}
6	T_6	12.66^{ab}	2.22^{abc}	1.86^{a}	2.77^{b}
7	T_7	13.22^{a}	1.66^{cd}	1.10^{b}	2.55^{b}
8	T_8	13.55^{a}	1.55^{d}	1.06^{b}	2.33^{b}
SE(±m)		0.36	0.21	0.17	0.19
C. D. at 5 % level		1.09	0.66	0.51	0.58

Table 3.5: Effect of growth regulators on shoot characters of *in vitro* culture of jack (5th subculture)

Sl. No.	Treatments	No. of days to shoot emergence	No. of shoot/explant	Shoot length (cm)	No. of leaves/shoot
1	T_1	10.77^{cd}	1.99^{b}	1.27^{bc}	2.55^{bcd}
2	T_2	11.11^{bc}	2.10^{ab}	1.43^{abc}	2.66^{bc}
3	T_3	9.77^{de}	2.33^{ab}	1.41^{abc}	2.88^{abc}
4	T_4	9.44^{e}	2.77^{a}	2.06^{a}	3.44^{a}
5	T_5	9.99^{cde}	2.77^{a}	1.12^{c}	3.10^{ab}
6	T_6	11.99^{ab}	2.44^{ab}	1.89^{ab}	2.98^{ab}
7	T_7	12.33^{a}	1.77^{b}	1.17^{c}	2.33^{cd}
8	T_8	12.88^{a}	1.77^{b}	1.04^{c}	2.00^{d}
SE(±m)		0.40	0.22	0.22	0.19
C. D. at 5 % level		1.21	0.68	0.68	0.58

Table 3.6: Effect of growth regulators on shoot characters of *in vitro* culture of jack (6th subculture)

Sl. No.	Treatments	No. of days to shoot emergence	No. of shoot/explant	Shoot length (cm)	No. of leaves/shoot
1	T_1	11.99^{ab}	1.88^{bc}	1.13^{b}	2.66^{bcd}
2	T_2	11.44^{bc}	2.10^{b}	1.17^{b}	3.03^{abcd}
3	T_3	10.86^{bc}	2.10^{b}	1.14^{b}	3.36^{abc}
4	T_4	10.55^{c}	2.22^{b}	2.08^{a}	3.84^{a}
5	T_5	10.99^{bc}	2.88^{a}	1.12^{b}	3.51^{ab}
6	T_6	12.66^{a}	2.21^{b}	1.42^{b}	3.44^{abc}
7	T_7	12.77^{a}	1.88^{bc}	0.97^{b}	2.55^{cd}
8	T_8	12.88^{a}	1.44^{c}	1.05^{b}	2.33^{d}
SE(±m)		11.99	0.22	0.16	0.30
C. D. at 5 % level		11.44	0.67	0.49	0.92

Table 3.7: Effect of growth regulators on shoot characters of *in vitro* culture of jack (7th subculture)

Sl. No.	Treatments	No. of days to shoot emergence	No. of shoot/explant	Shoot length (cm)	No. of leaves/shoot
1	T_1	11.44^{abcd}	2.00^{cd}	1.12^{b}	2.66^{cd}
2	T_2	11.88^{abc}	2.32^{bcd}	1.30^{b}	2.88^{bcd}
3	T_3	10.86^{cd}	2.55^{abcd}	1.25^{b}	3.00^{bcd}
4	T_4	10.44^{d}	2.88^{ab}	2.16^{a}	4.33^{a}
5	T_5	11.21^{bcd}	3.10^{a}	1.32^{b}	3.66^{ab}
6	T_6	11.66^{abc}	2.66^{abc}	1.38^{b}	3.22^{bc}
7	T_7	12.22^{ab}	1.99^{cd}	1.18^{b}	2.44^{cd}
8	T_8	12.44^{a}	1.88^{d}	1.07^{b}	2.10^{d}
SE(±m)		0.40	0.25	0.14	0.32
C. D. at 5 % level		1.21	0.78	0.44	0.99

Table 3.8: Effect of growth regulators on shoot characters of *in vitro* culture of jack (8th subculture)

Sl. No.	Treatments	No. of days to shoot emergence	No. of shoot/explant	Shoot length (cm)	No. of leaves/ shoot
1	T_1	11.22^{bcd}	2.21^{de}	1.15^{bc}	3.00^{bc}
2	T_2	12.10^{ab}	2.33^{cde}	1.30^{bc}	3.21^{bc}
3	T_3	10.88^{cd}	2.55^{bcd}	1.27^{bc}	3.33^{bc}
4	T_4	10.55^{d}	3.21^{ab}	2.26^{a}	4.44^{a}
5	T_5	10.99^{cd}	3.88^{a}	1.10^{bc}	3.88^{ab}
6	T_6	11.66^{abc}	3.00^{bc}	1.55^{b}	3.66^{ab}
7	T_7	12.32^{a}	1.88^{de}	1.03^{c}	2.99^{bc}
8	T_8	12.44^{a}	1.66^{e}	1.07^{bc}	2.55^{c}
SE(±m)		0.36	0.24	0.17	0.30
C. D. at 5 % level		1.09	0.73	0.52	0.92

4.2.2.1 Number of days to shoot emergence

A close examination of the data presented in Table 3.1-3.8 revealed that treatment T_4 took lesser days to shoot emergence followed by T_3 in all the subcultures and T_8 took more number of days to shoot emergence followed by T_7. All the treatments in the first subculture took more number of days to shoot emergence compared to other subcultures.

4.2.2.2 Number of shoot/explant

The effect of plant growth regulators on number of multiple shoot per explant are presented in Table 3.1-3.8. Number of multiple shoots counted in each subculture. In the first subculture T_6 recorded maximum number of shoots followed by T_5 and T_4.While remaining subcultures (second subculture to eighth subculture) T_5 produced more number of shoots per explant followed by T_4 and T_6 (Plate 5b). T_8 produced least number of shoots followed by T_7. Number of shoots increased with treatment T_4 after each subculture (2.38 to 3.88).

4.2.2.3 Shoot length (cm)

It is evident from the data presented in table 3.1-3.8 that maximum shoot length was observed in T_4 followed by T_6 in all the subcultures. Minimum shoot length was observed in T_8 followed by T_7 in all cases while in 6^{th} and 8^{th} subculture minimum shoot length was observed in T_7 followed by T_8.

4.2.2.4 Number of leaves/ shoot

Among the different treatment tried, number of leaves per explant showed significantly different values. The maximum number of leaves were recorded in T_4 followed by T_5 in all the subcultures. Number of leaves produced per shoot increased with each subculture. Initially T_4 produced 2.88 leaves which increased to 4.44 leaves in the eighth subculture. T_8 produced lesser number of leaves which was followed by T_7. There was no significant difference between treatments in the second subculture.

4.3 Effect of different concentrations of growth regulators and its combinations on rooting.

4.3.1 NAA and IBA

This experiment comprised of nine treatments. Half strength MS medium was used as basal medium. Plant growth regulators like NAA and IBA alone and their combination were used for rooting to find out the best medium for rooting.

4.3.1.1 Number of days to root emergence

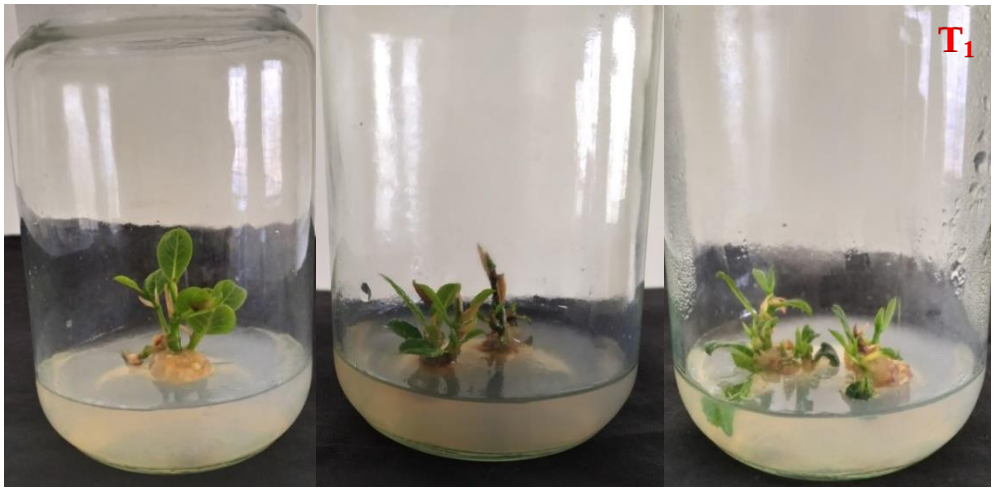
The multiplied shoots were kept in rooting medium for 3-4 months and all treatments failed to produce roots (Table 4.1).

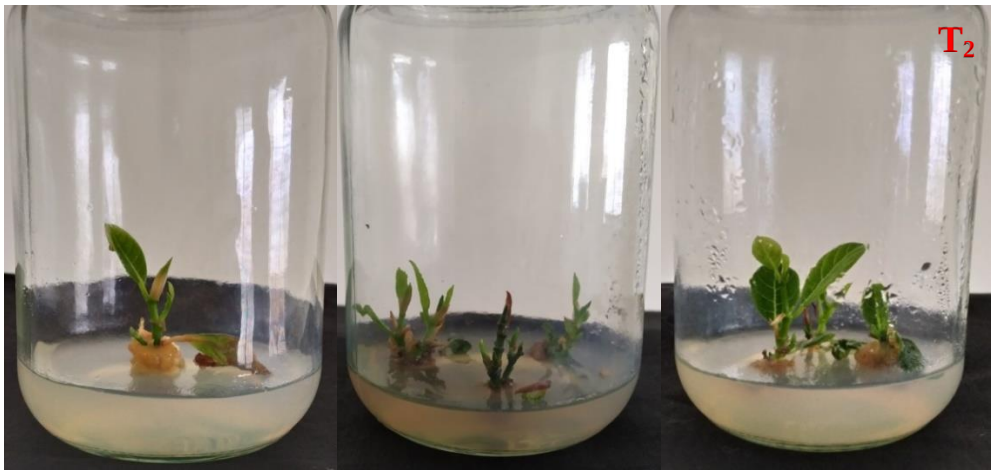
4.3.1.2 Number of roots/ shoot

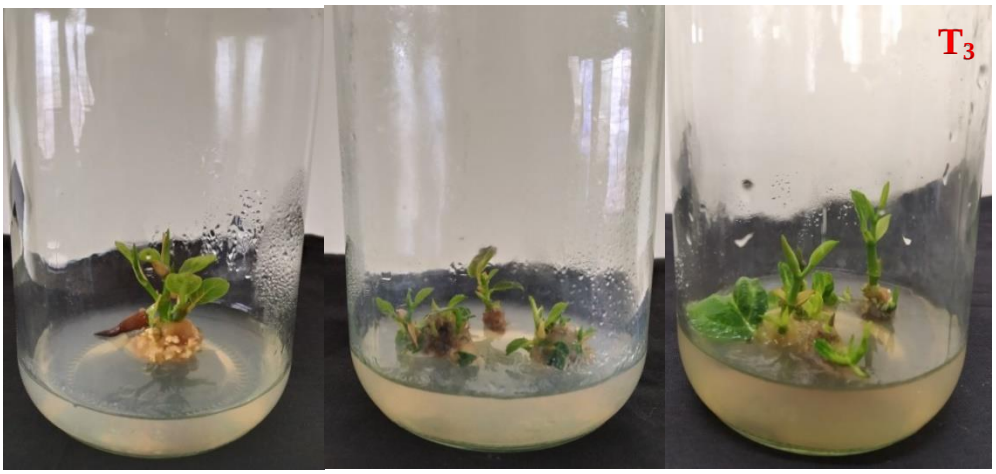
None of the treatments produced roots

4.3.1.3Root length (cm) / shoot

Observation was not made







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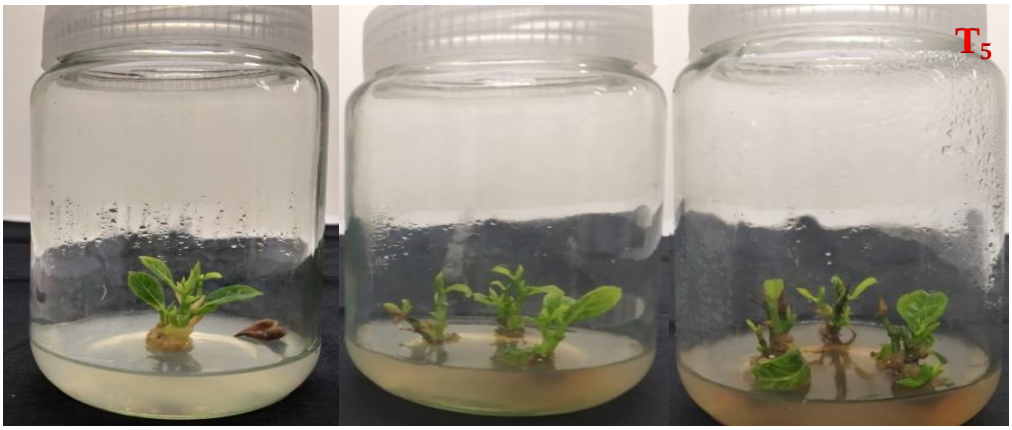

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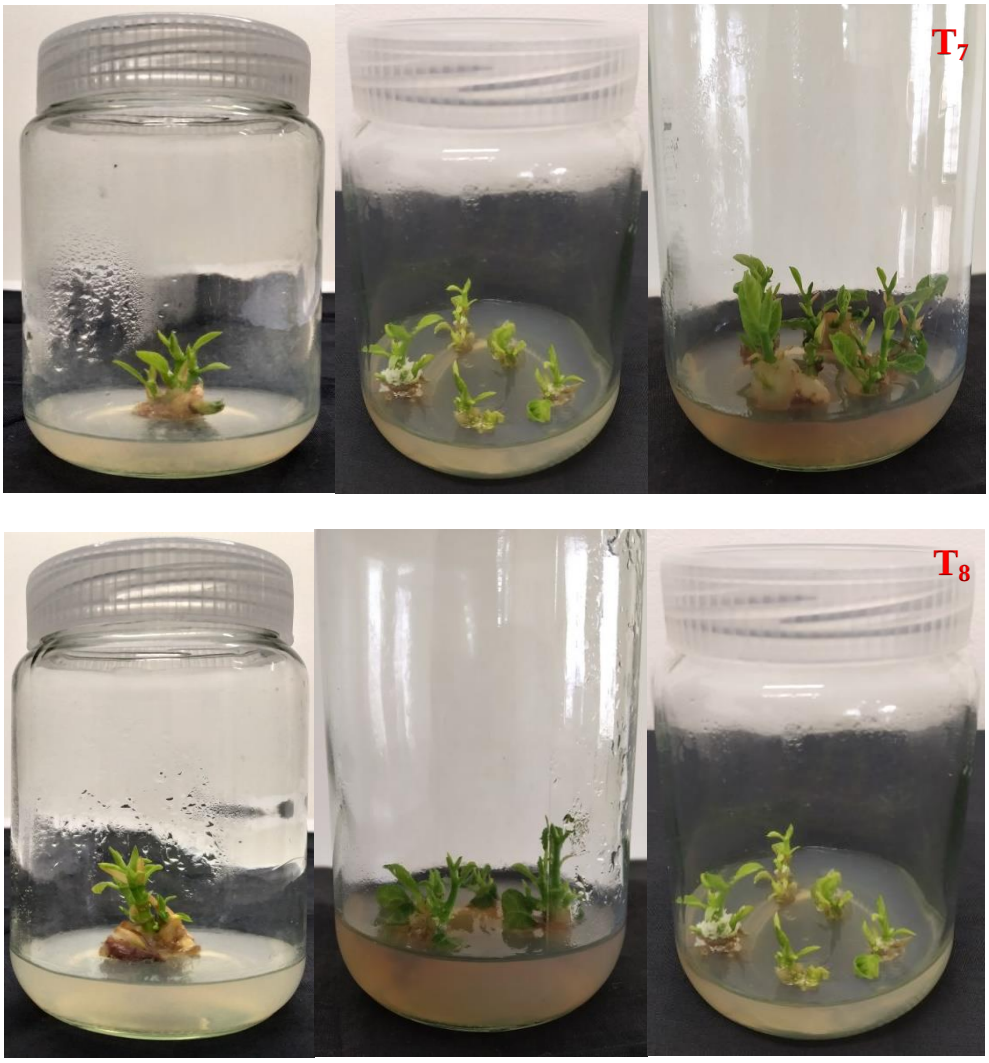


Plate 5b: Multiple shoots of *in vitro* cultured jackfruit

T_1 : MS medium + 1 mg/L BA + 0.35 mg/L GA_3

T_2 : MS medium + 1.5 mg/L BA + 0.35 mg/L GA_3

T_3 : MS medium + 2 mg/L BA + 0.35 mg/L GA_3

T_4 : MS medium + 2.5 mg/L BA + 0.35 mg/L GA_3

T_5 : MS medium + 3 mg/L BA + 0.35 mg/L GA_3

T_6 : MS medium + 3.5 mg/L BA + 0.35 mg/L GA_3

T_7 : MS medium + 4 mg/L BA + 0.35 mg/L GA_3

T_8 : MS medium + 4.5 mg/L BA + 0.35 mg/L GA_3

4.3.1.4 Number of secondary roots

This observation was also not counted (Plate 6a).

Table 4.1: Effect of different concentrations of growth regulators and its combinations on rooting.

Treatment s	No. of days to root emergence	No. of root/shoot	Root length (cm)/shoot	No. of secondary roots
T_1	–	–	–	–
T_2	–	–	–	–
T_3	–	–	–	–
T_4	–	–	–	–
T_5	–	–	–	–
T_6	–	–	–	–
T_7	–	–	–	–
T_8	–	–	–	–
T_9	–	–	–	–

T_1: ½ strength of selected basal medium
T_2: T_1 + 1 mg/L NAA
T_3: T_1 + 3 mg/L NAA
T_4: T_1 + 0.5 mg/L IBA
T_5: T_1 + 1 mg/L IBA
T_6: T_1 + 1 mg/L NAA + 0.5 mg/L IBA
T_7: T_1 + 1 mg/L NAA + 1 mg/L IBA
T_8: T_1 + 3 mg/L NAA + 0.5 mg/L IBA
T_9: T_1 + 3 mg/L NAA + 1 mg/L IBA

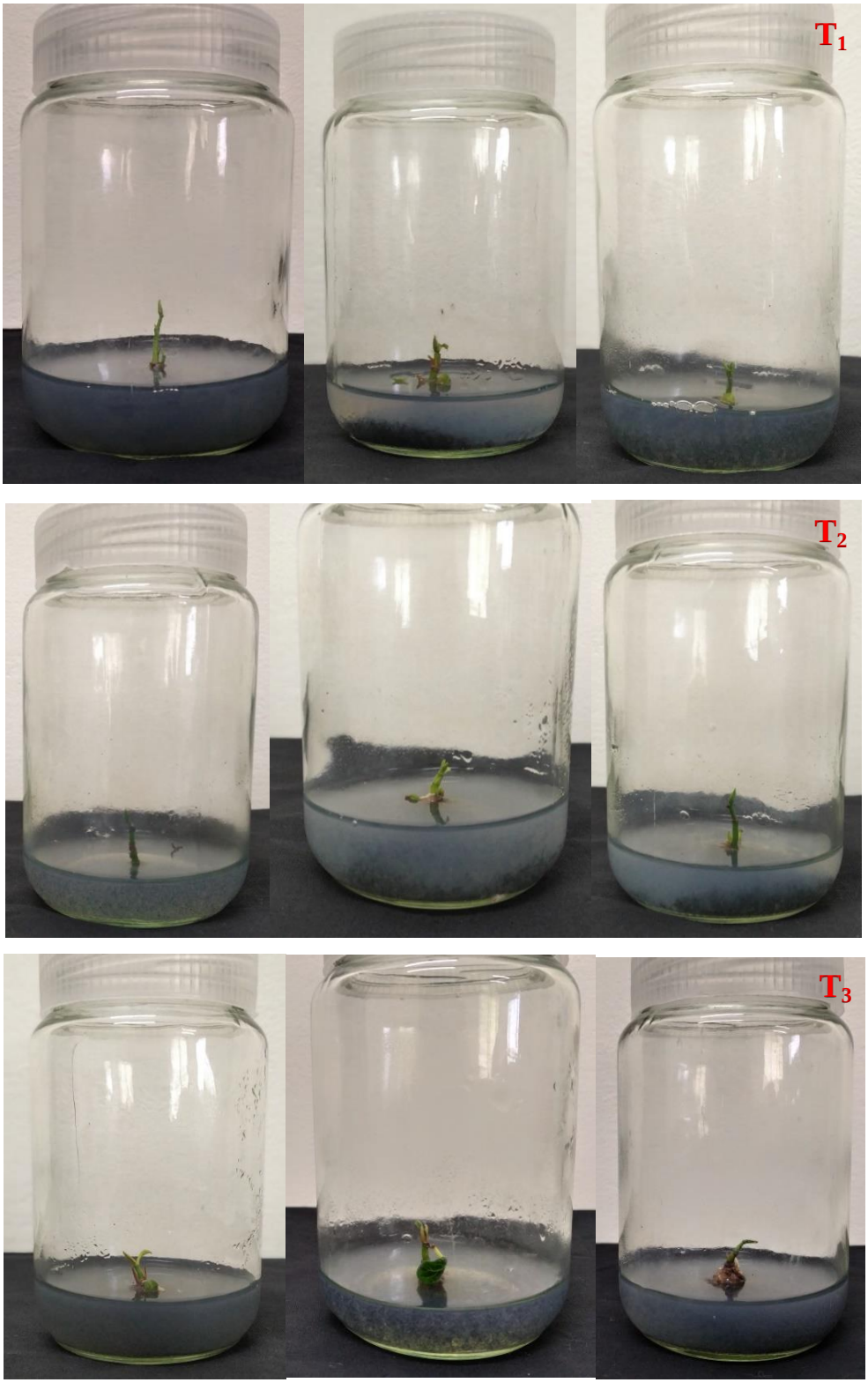
4.3.2 NAA, IBA, BA and GA_3

Table 3(b): Effect of different concentrations of growth regulators and its combinations on rooting.

Treatments	No. of days to root emergence	No. of root/shoot	Root length (cm)/shoot	No. of secondary roots
T_1	–	–	–	–
T_2	–	–	–	–
T_3	–	–	–	–
T_4	–	–	–	–
T_5	–	–	–	–
T_6	–	–	–	–
T_7	–	–	–	–
T_8	–	–	–	–

T_1: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA
T_2: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA
T_3: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 0.5 mg/L IBA
T_4: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L IBA
T_5: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA + 0.5 mg/L IBA
T_6: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA + 1 mg/L IBA
T_7: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA + 0.5 mg/L IBA
T_8: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA + 1 mg/L IBA

All the treatments were kept for more than three months but none of the treatments produced roots hence, observations were not made (Table 3b, Plate 6b).



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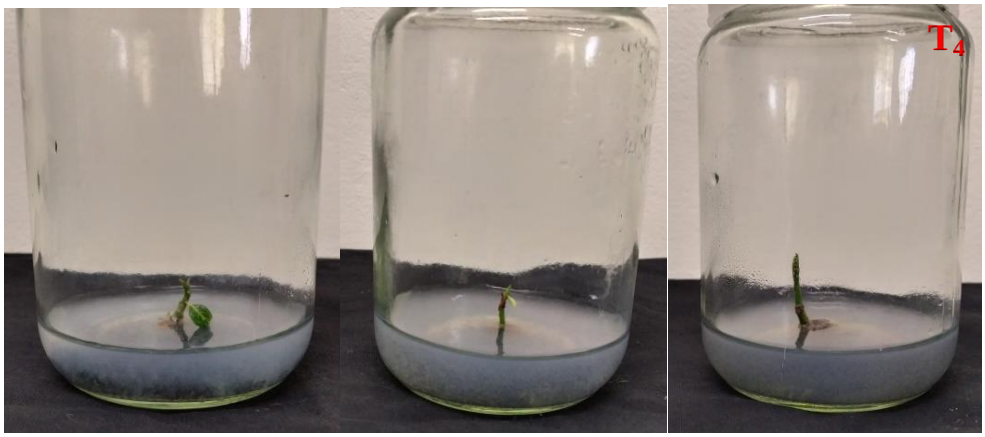

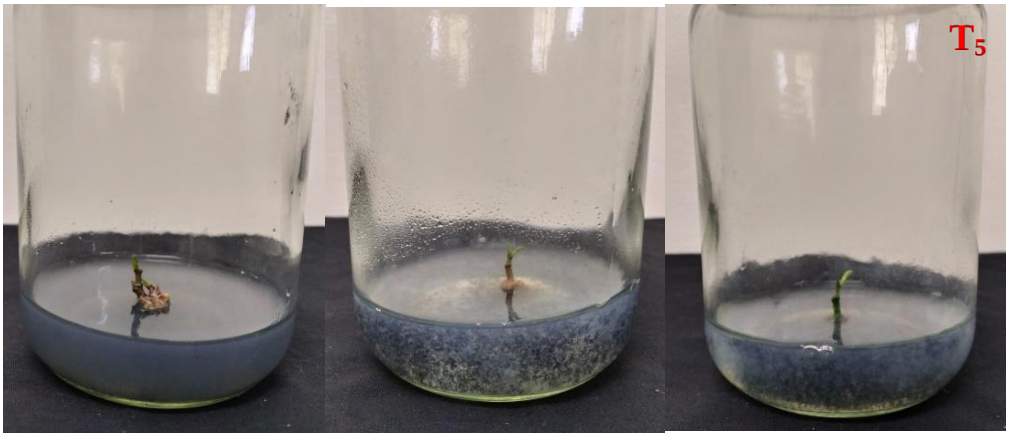

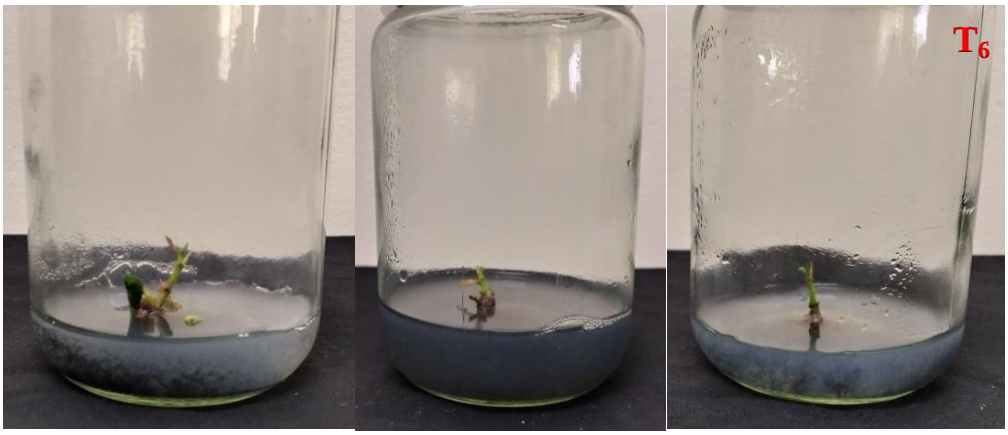

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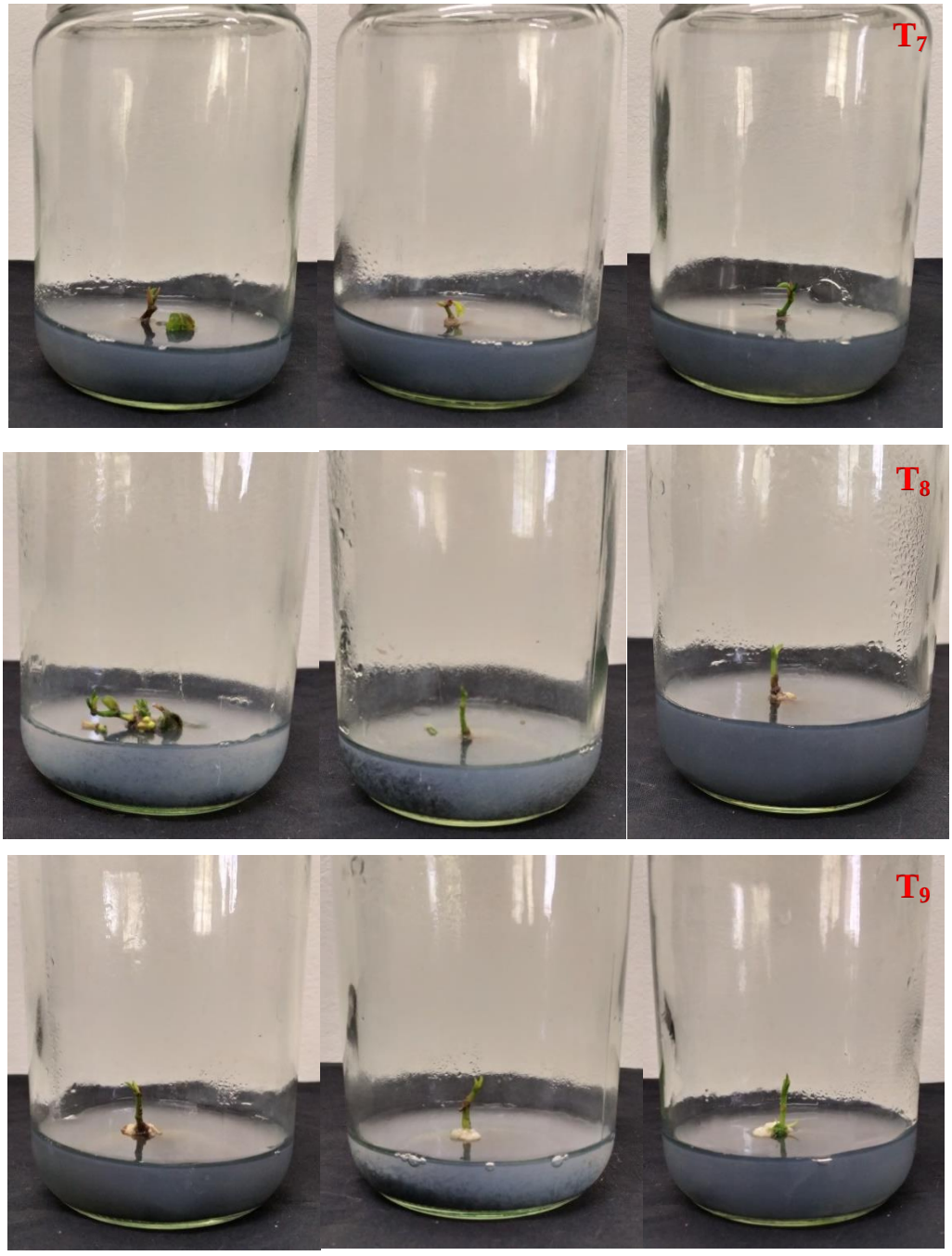

Plate 6a: Rooting stage of *in vitro* cultured jackfruit

T_1: ½ strength of selected basal medium	T_6: T_1 + 1 mg/L NAA + 0.5 mg/L IBA
T_2: T_1 + 1 mg/L NAA	T_7: T_1 + 1 mg/L NAA + 1 mg/L IBA
T_3: T_1 + 3 mg/L NAA	T_8: T_1 + 3 mg/L NAA + 0.5 mg/L IBA
T_4: T_1 + 0.5 mg/L IBA	T_9: T_1 + 3 mg/L NAA + 1 mg/L IBA
T_5: T_1 + 1 mg/L IBA	

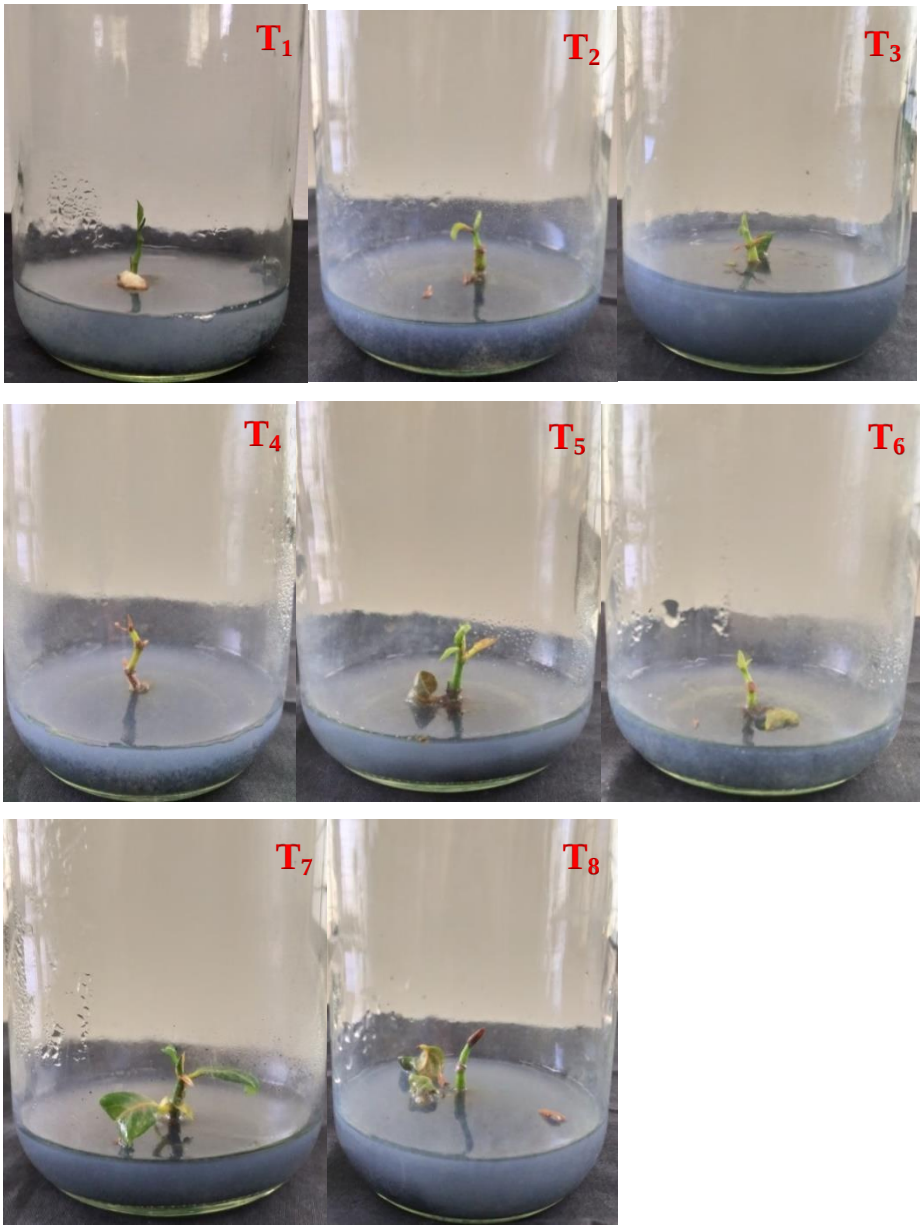

Plate 6b: Rooting stage of *in vitro* cultured jackfruit

T_1: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA

T_2: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA

T_3: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 0.5 mg/L IBA

T_4: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L IBA

T_5: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA + 0.5 mg/L IBA

T_6: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA + 1 mg/L IBA

T_7: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA + 0.5 mg/L IBA

T_8: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA + 1 mg/L IBA

Discussion

5. DISCUSSION

Jack fruit is an important humid zone tropical fruit crop of the world, which is valuable for its fruit and timber. Jack is propagated by seeds and vegetative parts. Commercially it is propagated by grafting methods. However, these methods are not much effective and requires more time to raise the seedlings (Hossain and Haq, 2006).

Micropropagation is one of the important tool for crop improvement through rapid propagation. With this importance present study was undertaken to develop a micropropagation protocol for jackfruit (*Artocarpus heterophyllus* Lam.). The results obtained are discussed in this chapter

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5.1 Effect of different media for the establishment of jackfruit

5.1.1 Growth parameters

Among the various media tried, MS medium fortified with 2 mg/L BA found to be significantly best with regard to number of days to shoot emergence, number of days to shoot multiplication and branching, shoot number per explant, shoot length and number of leaves (Figure 1, 2, 3). MS medium surpassed other media, this might be due to the higher concentration of nitrate, potassium and ammonium. And also in this medium nitrogen is supplied as ammonium and nitrate ions. This mixture of cation and anion balances the pH of the medium and also plays a significant role in plant growth.

Pierik, 1987 suggested that nutrient level in MS media is higher than the optimal plant growth, hence explant can absorb required amount of nutrients for their establishment and shoot proliferation. In MS media nitrogen is the prime component which influences plant growth and morphogenesis (Gamborg and Shyluk, 1970; Bayley *et al*., 1972; Wetherell and Dougall, 1976; Cousson and Van, 1993).

B_5 medium contains greater proportion of nitrate and potassium but lower concentration of ammonia. While in WPM ionic strength of salts are lesser than MS medium but sulphate is higher than the average. The amount of ammonium and nitrate in culture media has effective role in induction and differentiation of plant cell culture (Halperin and Wetherell, 1965; Wetherell and Dougall, 1976; Chaleff, 1983; Grimes and Hodges, 1990; Cousson and Van, 1993). Source of nitrogen and their proportion

in media will affect the production of endogenous cell metabolites, protein, hormones etc. (Preece, 1995). MS medium contains higher amount of NH_4NO_3 and KNO_3 that favours the establishment of shoot tip explant. This result was supported by Li and Han (2003) who reported that, formation of adventitious buds in fruit tree is mainly influenced by the ratio of NH_4NO_3 and KNO_3.

pH influnces the availability and uptake of nutrients and plant growth regulators, and also it affects the gelling efficiency of agar (Scholten and Pierik, 1998). Acidic pH favours the uptake of negatively charged ions, when the pH is increased uptake of positively charged ions occur. MS medium has slightly acidic pH of 5.68-5.69, when compared to B_5 and WPM media (5.75). pH might affect the uptake of ammonium and nitrate reported by Shibli *et al*. (1999).

Roy and Hadiuzzaman (1991) explained that a combination of BA and NAA favoured shoot proliferation up to 5.2±0.17 cm in *A. heterophyllus*. Azam and Rahmatullah (2009) reported that in jack MS medium is good for establishment. They also concluded that medium supplemented with 3.5mg/L BA showed higher frequency of sprouting and adventitious bud proliferation.

Season also influenced the survival of explant. Explants collected during December – January showed lowest microbial and fungal contamination. This might be due to the occurance of new flushes during this time and lesser atmospheric humidity. Shoot tip from mature tree has reduced the levels of fungal infection because shoot tip is covered by a protective sheath. This shoot tip sheath is intact during surface sterilization protecting the tip from harmful effect of mercuric chloride and it could be removed at the time of inoculation. Surface sterilization of explant with mercuric chloride minimized the fungal contamination. Shoot tip has less endogenous microorganism because it does not have a properly developed vascular system. Regenerative ability differed from cell to cell, tissue to tissue, organ to organ, species to species and cultivar to cultivar.

Similar results were found in Citrus and *Annona cherimola* (Duran *et al.,* 1989 and Jordan *et al.,* 1991).

Lee and Keng (2005) found that shoot explant cultured on MS medium supplemented with BA only resulted in more number of shoots/explant in jack. While

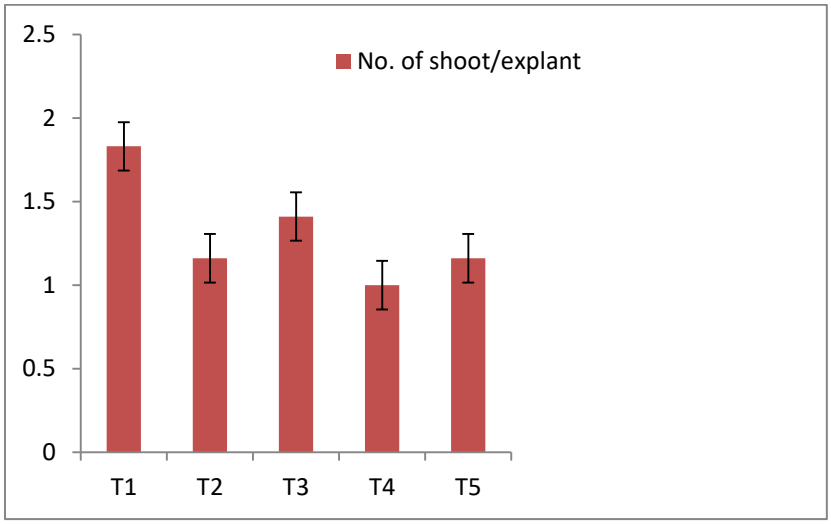

Figure 1: Effect of media on no. of shoot/explant

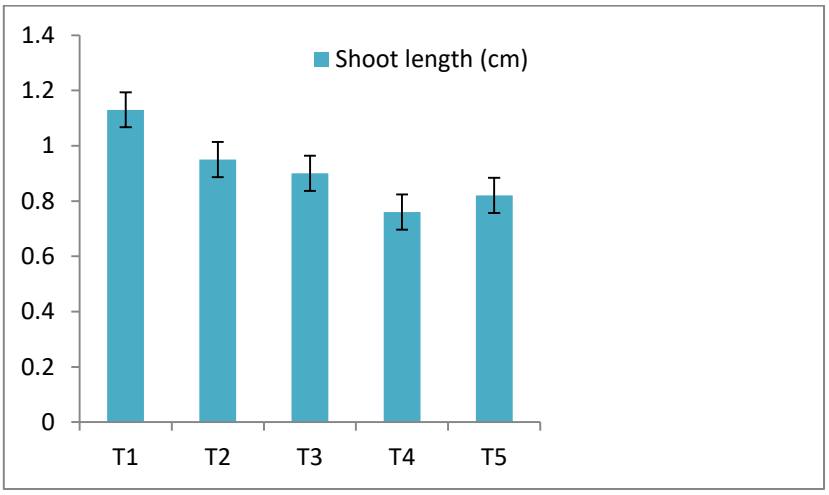

Figure 2: Effect of media on shoot length

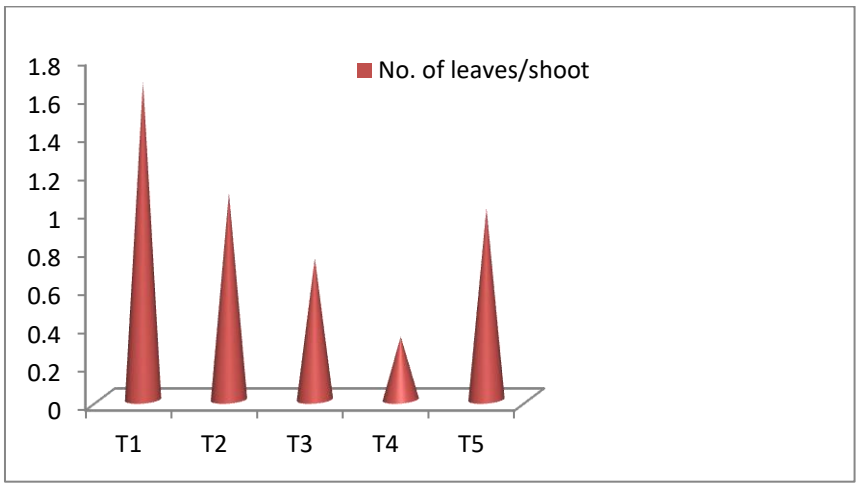

Figure 3: Effect of media on no. of leaves/shoot

Amany *et al*. (2007) reported that MS medium surpassed other nutrient medium such as B_5 and WPM with respect to average number of shoots/ explant in jack. They also concluded that MS medium fortified with 3 mg/L BA and 0.1 mg NAA produced the highest number of shoots, leaves/explant and shoot length and this result was little bit different from present study this might be due to the genetic factors.

Jaiswal and Narayan (1985) demonstrated that maximum shoot number through stem in MS containing 2 mg/l BA in jack. MS medium fortified with 1 mg/L BA and 0.5 mg/L kinetin produced 5-7 shoots from each explant with in four weeks (Roy *et al*., 1990) in jack and in the present study the concentration of BA used was twice the amount used by them. This might be due to the different cultivar used in the study and also due to the age of the mother plant. *In vitro* propagation of *Syzygium francisii* produced large number of *in vitro* plantlet (Shatnawi *et al*., 2004). They found that among the different cytokinin tested MS medium supplemented with BA was superior with respect to shoot length and number of new shoots per explant.

5.2 shoot multiplication

5.2.1 Effect of different concentrations of growth regulator BA with best medium from the 1st experiment, for shoot multiplication

5.2.1.1 Plant growth parameters

Various growth parameters were recorded during shoot multiplication. Treatment T_3 (T_2 + 1 mg/L BA) was found to be significantly superior treatment in terms of number of days to shoot emergence, number of shoot/ explant and number of leaves/ shoot. The treatment T_2 had highest shoot length. Treatment T_1 showed poor response in all the growth characters.

The medium devoid of cytokinin gave least average number of shoots which indicates strong apical dominance. Highest mean number of shoot obtained in this study was in 3 mg/L BA (T_2 + 1 mg/L BA). In jackfruit, Lee and Keng (2005) observed 4.5 mg/L BA showed maximum number of shoot/ explant. The findings of Adiga (1996) revealed that BAP at 2 mg/L showed better response in terms of shoot multiplication, length of shoot, and number of leaves per explants. The difference might be due to the different cultivars used in these studies which produce different responses.

5.2.2 Effect of different concentrations of growth regulator BA and Gibberelic acid (GA_3) with best medium from the 1st experiment, for shoot multiplication.

5.2.2.1 Plant growth parameters

While assessing the impact of treatments on number of days to shoot emergence, it could be seen that the treatment T_4 was the best in terms of number of days to shoot emergence in all the subcultures while T_8 took more number of days to shoot emergence which was followed by T_7.

T_6 recorded more number of multiple shoots in first subculture while T_5 had maximum number of multiple shoots in the remaining subculture. After each subculture number of shoots were increased. Highest shoot length and maximum number of leaves were observed on T_4 in all the subculture where as T_7 and T_8 showed poor response in all the growth parameters (figure 4, 5, 6). Darwesh *et al.* (2014) who reported that control treatment (in the absence of plant growth regulator) significantly produced shortest length of shoots in fig and MS medium with 5 mg/L BA and 1 mg/L GA_3 recorded the highest shoot elongation in fig. Demiralay *et al*. (1998) reported that cytokinin has significant role in culture medium for shoot induction and proliferation in fig. This was more or less similar to the findings of Nobre *et al.* (1998); Kumarm *et al.* (1998); Fraguas *et al*. (2004); Shahcheraghi and Shekafandeh (2016); Vieitez and Vieitez, (1980); Park *et al*. (2008).

The major problem faced in the present study was exudation of phenolics in the medium and this was minimized to a certain limit by means of frequent subculturing. A study conducted by Co *et al.* (2009) they stated that *in vitro* culture of majority of woody plants and herbaceous plants causes medium browning, when the browning was maximum, the explant become black or brown in colour and finally it become necrotic leading to death of the explant. Çördük and Aki (2011) described that browning was mainly due to phenolic exudation by the explant which was oxidized and become phytotoxic hence a detailed investigation is required before inoculating the explant on culture medium.

Elmore *et al.* (1990); Liu and Yang (2011) described that after wounding, majority of hardwood species will form phenolic compounds and these phenolic

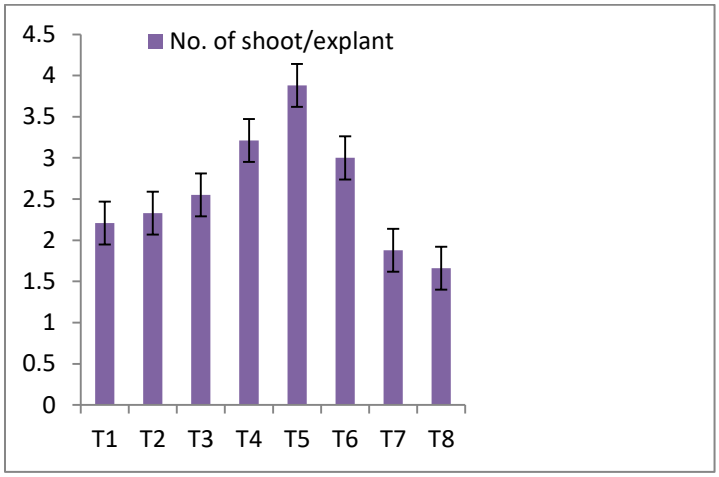

Figure 4: Effect of plant growth regulator on no. of shoot/explant

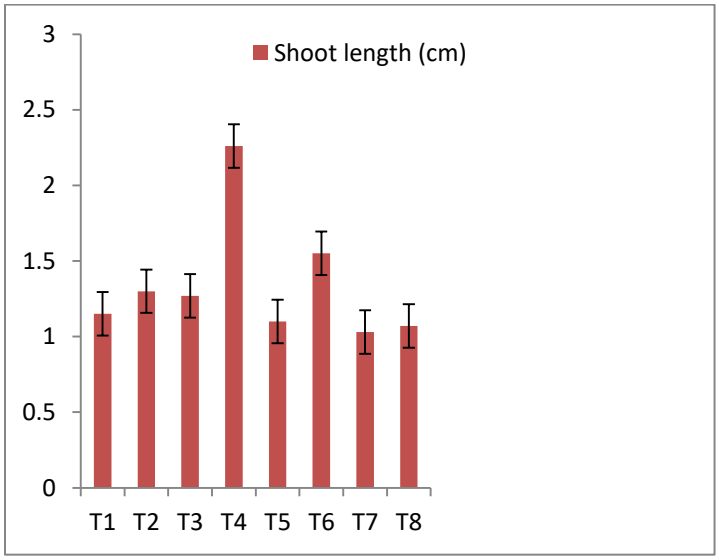

Figure 5: Effect of plant growth regulator on shoot length

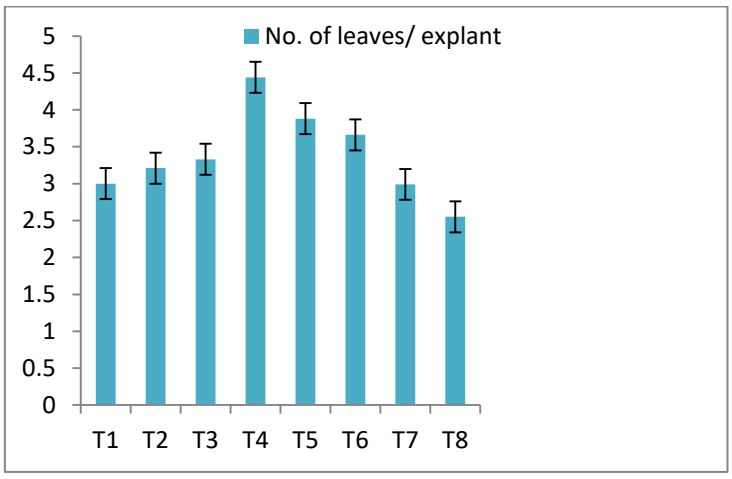

Figure 6: Effect of plant growth regulator on no. of leaves/explant

substances converted to quinines as a result of oxidation which causes tissue blackening and inhibit the *in vitro* plant responses like morphogenesis and the phenolics could be reduced by keeping the explants in antioxidant solution containing citric acid and ascorbic acid from the time of collection of explants to inoculation. Roy *et al*. (1993) suggested that rainy season was the ideal time for collection of explant at this time phenolic exudation was very low. Degree of browning depends on tissue age, season of culture initiation, medium composition and also it varies from species to species (Sáenz *et al*., 2010).

Torresm, (2013) described that BA, synthetic cytokinin which has effective role in shoot proliferation compared to other cytokinins. During tissue culture plant growth regulator respond varyingly from species to species and also it depends on ability of tissue and type of plant growth regulator used (Singh *et al.,* 2016). Shatnawi *et al*. (2019) reported that enhancement of shoot multiplication may be due to the interaction between BA and IBA which induced shoot formation because of increased meristematic activity.

Comlekcioglu *et al*. (2007) reported that culture medium containing 0.2 mg/L GA_3 and 0.5 mg/L BA resulted highest in culture establishment in fig. Xiao *et al*. (2016) proved positive effect of GA_3 on shoot elongation and cell division in fig. Similar results were obtained by Inthima *et al*. (2017). This was in agreement with Hesami *et al.* (2018) who reported that increased shoot length was obtained in *Ficus religiosa* by culturing the shoot tips on MS medium fortified with 0.5 mg/L GA_3 and 0.5 mg/L BA.

Stuart and Street, (1971) suggested that GA_3 at low concentration is essential for culturing medium and it often produces the effects similar to those of auxin. From Amin and Jaiswal, (1993); Islam *et al*. (1997); Amin *et al*. (2001); Rahman *et al*. (2004); reported that cytokinin free medium gave least number of shoots because of strong apical dominance. Production of axillary shoot favoured by BA and Kn. BA is more effective than Kn in terms of multiple shoot production in *Artocarpus heterophllys, Ficus religiosa, Azadirachta indica, Ficus benghalensis.* Eglal *et al.* (2015) recorded highest shoot multiplication of jack in the medium supplemented with 2.0 mg/L BA and 0.5 mg/L Kn and they found that higher levels of BA (5.0 mg/L)

reduced multiplication of shoots.

Axillary shoot proliferation were observed on MS medium containing BA this might be due to the role of cytokinin in overcoming apical dominance in Ipomoea (Cline *et al*., 1997). Cytokinin plays an important role in stimulating the cell division, releasing the lateral bud dormancy, promoting adventitious bud formation and growth of lateral buds and also it controls cell cycle (Melara and Arias, 2009). In pomegranate MS medium fortified with 1 mg/L BA and 1 mg/L kinetin with 3 per cent sucrose gave higher number of shoot and shoot length (Singh, 2014). Gayathri and Sathyanarayana (2015) who reported MS medium supplemented with 2 mg/L BA produced more number of shoots and leaves, highest shoot length in jack but in the present study BA concentration ranges from 2.5-3 mg/L gave more number of multiple shoot, more number of leaves and highest shoot length. This variation might be due to the difference in genetic factor of the variety used in the present study. Miro and Acedo (2015) were identified 3 mg/L BA, 0.5 mg/L GA_3 and 0.1 mg/L IBA resulted taller multiple shoots and had more number of nodes and leaves in jack. Gogoi (2017) found that BA is an effective plant growth regulator for shoot organogenesis while lower concentration requires longer period for bud break and higher concentration leads to fleshy leaves and stem and micro shoots cultured on MS medium containing GA_3 1.5 mg/L showed optimum shoot elongation in *Morus alba.*

5.3 Effect of different concentrations of growth regulators and its combinations on rooting.

No rooting was observed in all the treatments tried with various concentrations and combinations of growth regulator. Success rate of micropropagation of woody perennial is limited due to contamination, phenolic exudation, vitrification, low induction of rooting and acclimatization etc. one of the reason for absence of rooting in present study might be the formation of basal callus which was observed in in most of the cultures. Callus affect the rooting competence of microplants as it interfere with the physiological process by trapping the growth constituents like auxin and also act as a physical barrier. Accumulation of inhibitory substance like phenolic compound in the culture medium is a major problem in rooting (Vengadeshan *et al.,*

2002). Nandwani *et al*. (2004) described that rooting is a difficult phenomenon in micropropagation of tree species.

Amin (1992) found that 83% rooting efficiency in jackfruit when microshoots cultured on MS medium with IBA and NAA at 2 mg/L each and 68% rooting with IBA 2 mg/L. Half strength MS medium supplemented with IBA and IAA 1 mg/L each produced 90% rooting in jackfruit (Islam *et al.,* 1993). Roy and Roy (1996) reported that synergistic effect of IBA and NAA found to be ideal for *in vitro* rooting in Jackfruit. MS medium containing 1.0 mg/l of both IBA and NAA gave the highest rooting percentage (80%), average number of roots/explant (4.3) and average root length (4.0 cm) (Harb *et al.,* 2015). An increasing concentration of IBA and NAA increases the quantity of rooting while further increasing concentration to a higher level decreases rooting in jackfruit (Kamal, 2018).

Summary

6. SUMMARY

The present study entitled, “Development of micropropagation protocol for jackfruit (*Artocarpus heterophyllus* Lam.)” was conducted during 2018-2020. The salient observations of the study are summarized in this chapter.

Objective of the research was to establish a micropropagation protocol for jackfruit (*Artocarpus heterophyllus* Lam.) using shoot tip explants. The main aim was to know the best medium for culture establishment, best combination of plant growth regulators for shoot multiplication and rooting of KJ 182 jack. The research was conducted at tissue culture laboratory, Regional Agricultural Research Station, Pilicode, Kasaragod. Three experiments were conducted which included effect of different media for the establishment of jackfruit, effect of combination of plant growth regulators like BA and GA_3, effect of different concentrations of growth regulators and their combinations on rooting. The experiments were carried out with completely randomized design with five treatments replicated with four times. In the second experiment was with five treatments and four replications and also another combination of growth regulators were tried for shoot multiplication with eight treatments replicated thrice. Third experiment comprised of nine treatments and three replications. Apart from these treatments another rooting experiment was carried out with eight treatments replicated thrice. Experimental material used was shoot tip explant of jack KJ 182 located at Chirapuram near Nileshwar.

The first experiment for culture establishment consisted of five treatments such as T_1: MS medium, T2: ½ MS medium, T3: Modified MS medium (Concentration of macro elements was made into half), T4: Woody plant medium, T5: Gamborge B_5 medium. 2 mg/L BA was added to each treatment.

In all the treatments tried only treatment T_1 had shown the best result with respect to growth parameters (took minimum days to shoot emergence, multiplication and branching). T_4 and T_5 showed poor results for all the growth parameters compared with other treatments.

Shoot multiplication study was conducted with five treatments. The best treatment obtained from the first experiment was used for the shoot multiplication

experiment. Treatments include T_1: Selected basal medium, T_2: Best medium from the 1st experiment, T_3: T_2 + 1 mg/L BA, T_4: T_2 + 2 mg/L BA, T_5: T_2 + 3 mg/L BA.

Treatment T_3 had shown better result for all the parameters except in shoot length. T_2 had highest shoot length and T_1 showed poor response for all the growth characters.

Apart from the above treatments another shoot multiplication experiment was carried out with eight treatments. The treatments used were T_1 : Best medium from the first experiment (MS medium) + 1 mg/L BA + 0.35 mg/L GA_3, T_2 : MS medium + 1.5 mg/L BA + 0.35 mg/L GA_3, T_3 : MS medium + 2 mg/L BA + 0.35 mg/L GA_3, T_4 : MS medium + 2.5 mg/L BA + 0.35 mg/L GA_3, T_5 : MS medium + 3 mg/L BA + 0.35 mg/L GA_3, T_6 : MS medium + 3.5 mg/L BA + 0.35 mg/L GA_3, T_7 : MS medium + 4 mg/L BA + 0.35 mg/L GA_3, T_8 : MS medium + 4.5 mg/L BA + 0.35 mg/L GA_3.

Among the different treatments tried for shoot multiplication, it was clear that T_4 took minimum days to shoot emergence which was followed by T_3 in all the subcultures. With respect to number of shoot/explant, T_5 produced more number of shoots per explant followed by T_4 and T_6 while in the first subculture T_6 recorded maximum number of shoots. In the case of shoot length maximum length of shoot was observed on T_4 followed by T_6 in all the subcultures. T_4 recorded maximum number of leaves followed by T_5 in all the subcultures. After each subculture number of shoot, shoot length and leaves were increased. T_8 and T_7 had shown poor response for all the growth parameters.

The last experiment was on rooting. This experiment comprised of nine treatments like T_1: ½ strength of selected basal medium, T_2: T_1 + 1 mg/L NAA, T_3: T_1 + 3 mg/L NAA, T_4: T_1 + 0.5 mg/L IBA, T_5: T_1 + 1 mg/L IBA, T_6: T_1 + 1 mg/L NAA + 0.5 mg/L IBA, T_7: T_1 + 1 mg/L NAA + 1 mg/L IBA, T_8: T_1 + 3 mg/L NAA + 0.5 mg/L IBA, T_9: T_1 + 3 mg/L NAA + 1 mg/L IBA.

Multiplied shoots were cultured on rooting medium containing activated charcoal for 4 months. But all the treatments failed to produce roots.

Another rooting experiment was done with eight treatments. Treatments were T_1: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA, T_2: MS + 2.5 mg/L BA + 0.35

mg/L GA_3 + 3 mg/L NAA, T_3: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 0.5 mg/L IBA, T_4: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L IBA, T_5: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA + 0.5 mg/L IBA, T_6: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA + 1 mg/L IBA, T_7: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA + 0.5 mg/L IBA, T_8: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA + 1 mg/L IBA.

All the treatments were kept under dark for three months but none of the treatments produced roots.

MS medium with 2 mg/L BA is best for culture establishment in jack. A combination of 2.5-3 mg/L BA and 0.35 mg/L GA_3 in MS medium is required for the successful shoot multiplication in jack.

References

7. REFERENCES

Adiga, D.J. 1996. Clonal Propagation of Jackfruit (*Artocarpus heterophyllus* Lam) cv. Singapore Jack through tissue culture. *Curr.Sci*. 78: 1231- 1234.

Ajeesh, B.R. 2018. Field evaluation of promising jackfruit (*Artocarpus heterophyllus* Lam.) types. MSc Hortic. Thesis, KAU, Thrissur.

Ali, J., Bantte, K. and Feyissa, T. 2016. Protocol optimization for *in vitro* shoot multiplication of Jackfruit (*Artocarpus heterophyllus* L.). *African J. Biotechnol.* 16(2): 87-90.

Amany, H.M., Ali, E.A., and Boshra, E.S. 2007. *In vitro* propagation of jackfruit (*Artocarpus heterophyllus* L.). *J. Appl. Sci. Res.* 3(3): 218-226.

Amany, H.M., Enas, A.M., Ali., and Boshra, E.S. 2007. *In vitro* propagation of Jackfruit (*Artocarpus heterophyllus* Lam.). *J. Appl. Sci. Res.*, 3(3): 218-226.

Amin, M.N. 1992. *In vitro* rooting of Jackfruit (*Artocarpus heterophyllus*) microcuttings derived from mature trees. *Plant Tissue Cult*. 2:129-133.

Amin, M.N. and Jaiswal, V.S. 1993. *In vitro* response of apical bud explants from mature trees of jackfruit (*Artocarpus heterophyllus*). *Plant Cell Tissue Organ cult.* 33(1): 59-65.

Amin, M.N., Habib, M.A., Azad, M.A.K., and Akhteruzzaman, M. 2001. Large Scale Plant Regeneration in *Ficus religiosa* L through tissue culture. *4 Int. Conf. (1-3 Nov, Dhaka) Plant Tissue Cult*, p.9.

Bayley, J.M., King, J., and Gamborg, O.L. 1972a. The effect of the source of inorganic nitrogen on growth and enzymes of nitrogen assimilation in soybean and wheat cells in suspension cultures. *Planta.*105: 15- 24.

Bayley, J.M., King, J., and Gamborg, O.L. 1972b. The ability of amino compounds and conditioned medium to alleviate the reduced nitrogen. *Planta.* 105: 25-32.

Beversdorf, W.D. 1990.Micropropagation in crop species. In: Nijkamp, H.J., Plas, V.D., and Aartrijk J.V (eds), *Progress in Plant Cellular and Molecular Biology*. Kluwer Academic Publishers, Dordrecht, pp. 3-12.

Borchetia, S., Das, S.C., Handique, P.J., and Das, S., 2009. High multiplication frequency and genetic stability for commercialization of the three varieties of

micropropagated tea plants (*Camellia spp*.). *Sci. Hortic.*, 120(4): 544-550.

Bose, T.K. 1985. Jackfruit. In: Mitra, B.K. (ed.), *Fruits of India: Tropical and Subtropical*. NayaProkash, Calcutta, India, pp. 487-497.

Chaleff, R.S. 1982. Induction, maintenance, and differentiation of rice callus cultures on ammonium as sole nitrogen source. *Plant Cell Tissue Organ Cul.* 2(1): 29-37.

Cline, M., Wesse, T., and Iwamura, H. 1997. Cytokinin/auxin control of apical dominance in *Ipomoea* nil. *Plant Cell Physiol.* 38(6): 59-667.

Comlekcioglu, S., Kuden, A.B., Kacar, Y.A., and Kamberoglu, M.A. 2007. Meristem culture of two fig cultivars in Turkey. *Fruits*, 62: 125–131.

Çördük, N. and Aki, C. 2011. Inhibition of browning problem during micropropagation of *Sideritistro janabornm*., an endemic medicinal herb of Turkey. *Rom Biotechnol. Lett.* 16: 6761-6765.

Cousson, A. and Van, K.T.T. 1993. Influence of ionic composition of the culture medium on de novo flower formation in tobacco thin cell layers. *Can. J. Bot*. *71*(3): 506-511.

Dalal, M.A., Das, B., Sharma, A.K., Mir, M.A., and Sounduri, A.S. 2006.*In vitro* cloning of apple (*Malus domestica* Borkh) employing forced shoot tip cultures of M9 rootstock. *Indian J. biotechnol*. 5: 543-550.

Darwesh, H.Y., Bazaid, S.A., and Samra, B.N.A. 2014.*In vitro* propagation method of *Ficuscarica* at Taif governorate using tissue culture technique. *Int. J. Adv. Res.* 2(6): 756-761.

Debergh, P.C. and Read, P.E. 1991. Debergh P.C. and Zimmerman, R.H. (eds), *Micropropagation–Technology and Application*.Kluwer Academic Publishers, Netherlands. 512 p.

Demiralay, A., Yalcin-Mendi, Y., Aka-Kacar, Y., Centime, S., Aksoy, U., Ferguson, L., and Hepaksoy, S. 1998. *In vitro* propagation of *Ficus carica* L. var. Bursa Siyahi through meristem culture. *Acta Hortic*. 480: 165–167.

DES GOK [Department of Economics and Statistics, Government of Kerala]. 2016. Economics and statistics department home page [on-line]. Available: http://www.ecostat.kerala.gov.in/index. php/agriculture. [24 June 2018].

Duran, V.N., Ortegu, V., and Nauarso, L. 1989.Morphogenesis and tissue culture of three citrus species. *Plant Cell Tissue Organ Cult.* 16: 123-133.

Dutton, P.R. 1976. *Artocarpus heterophyllus*-Jackfruit. In: Garner, R.J. and Chaudhuri, S.A. (eds), *The Propagation of Tropical Fruit Trees*. FAO, CAB, London, pp. 269-290.

Elmore, H.W., Samples, B., Sharma, S., and Harrison, M. 1990. Influence of cultural and physiochemical factors on ascorbate stability in plant tissue culture media. *Plant cell, tissue and organ cult.* 20(2): 131-135.

El-Zaher, M.H.A. 2008. Studies on micro propagation of jackfruit 1-Behaviour of the jackfruit plants through the micropropagation stages. *World J. Agric. Sci.* 4 (2): 263-279.

Fraguas, C.B., Pasqual, M., Dutra, L.F., and Cazetta, J.O. 2004.Micropropagation of fig (*Ficus carica* L.)'Roxo de Valinhos' plants. *In Vitro Cell. Dev. Biol Plant*. 40(5): 471-474.

Galaverna, G., Silvestro, G., Cassano, A., Sforza, S., Doceana, A., Drioli, E., and Marchelli, R. 2008. A new integrated membrane process for the production of concentrated blood orange juice: effect on bioactive compounds and antioxidant activity. *Food Chem.* 106: 1021–1030.

Gamborg, O.L. and Shyluk, J.P. 1970. The culture of plant cells with ammonium salts as a sole nitrogen source. *Plant Physiol*. 45: 598-600.

Gayathri, R. and Sathyanarayana, B.N. 2015. *In vitro* culture of three elite clones of jack fruit. *The Bioscan* 10(2): 713-717.

Gogoi, G., Borua, P.K., and Al-Khayri, J.M. 2017. Improved micropropagation and *in vitro* fruiting of *Morus indica* L.(K-2 cultivar). *J. Genet. Eng. Biotechnol.* 15(1): 249-256.

GOI [Governemt of India]. 2018. *Horticultural statistics at a glance 2018* [on-line]. Available:

http://agricoop.nic.in/sites/default/files/Horticulture%20Statistics%20at%20a%20Glance-2018.pdf [20 october 2019]

Goswani, C., Hossain, M.A., Mortuza, M.G., and Islam, R. 2010. Physicochemical parameters of Jackfruit seeds in different growing areas. *Int. J. Bioresearch* 2(10): 01- 05.

Grimes, H.D. and Hodges, T.K. 1990. The inorganic NO3-: NH4+ ratio influences plant regeneration and auxin sensitivity in primary callus derived from immature embryos of indica rice (*Oryza sativa* L.). *J. Plant Physiol*. 136(3): 362-367.

Halperin, W. and Wetherell, D.F. 1965.Ammonium requirement for embryogenesis *in vitro. Nat. 205*(4970): 519-520.

Haq, N. 2006. Jackfruit, *Artocarpus heterophyllus* L. International Centre for underutilized crops. Southampton, United Kingdom, 20p.

Harb, E.M., Alhady, M.R.A.A., and Elsalam, N.A.A. 2015. *In vitro* rapid propagation of jackfruit (*Artcarpus heterophyllus* Lam.). *Am. Eurasian J. Agric. Environ. Sci.* 15(2): 147-153.

Hasan, M.K., Ahmed, M.M., and Miah, M.G., 2008. Agro-economic performance of jackfruit-pineapple agroforestry system in Madhupur tract. *J. Agric. Rural Dev.* 6(1): 147-156.

Hesami, M., Daneshvar, M.H., Yoosefzadeh-Najafabadi, M., and Alizadeh, M. 2018. Effect of plant growth regulators on indirect shoot organogenesis of *Ficus religiosa* through seedling derived petiole segments. *J. Genet. Eng. Biotechnol.* 16(1): 175-180.

Hossain, A.K. and Haq, N. 2006. Jackfruit, *Artocarpus heterophyllus*. Field manual for extension workers and farmers. Southampton, United Kingdom, 27p.

Inthima, P., Nakano, M., Otani, M., Niki, T., Nishijima, T., Koshioka, M., and Supaibulwatana, K. 2017. Overexpression of the gibberellin 20-oxidase gene from *Torenia fournieri* resulted in modified trichome formation and terpenoid metabolities of *Artemisia annua* L. *Plant Cell Tissue Organ Cult.* 129(2): 223-236.

Islam, M.S., Sen, J., Alam, N., and Roy, S.K. 1993. Propagation of jackfruit (*Artocarpus heterophyllus*) through zygotic embryo culture *in vitro* [short communication-in Bangladesh]. *Plant Tissue Cult.* 3:51-55.

Islam, R., Hoque, A., Khalekuzzaman, M., and Joarder, O.I. 1997.Micropropagation of *Azadirachta indica* A. Juss.from explants of mature plants. *Plant Tissue Cult*. 7: 41-46.

Jaiswal, V.S. and Narayan, P. 1985. Regeneration of plantlets from the callus of stem segments of adult plants of *Ficus religiosa* L. *Plant Cell Rep.* 4(5): 256-258.

James, D.J. 1988. Cell and tissue culture technology for the genetic manipulation of temperate fruit trees. In: Russell, G.E. (ed.), *Biotechnology of higher plants*. Intercept. Dorset, England, pp. 33-80.

Jordan, M., Iturriaga, L., Roveraro, C., and Goreux, A. 1991.Promotion of *Annona cherimola in vitro* shoot morphogenesis as influenced by antioxidants. *Gartenbauwissenchaft*.56: 224-227.

Kamal, N.H.M., Aziz, M.A., Kadzimin, S., and Rashid, A.A. 2018. *In vitro* mass multiplication of *Artocarpus heterophyllus* Lam var. Tekam Yellow. *Pertanika J. Trop. Agric. Sci.* 41(3): 1289 – 1314.

Khan, F.R., UR-Rahman, H., Abbasi, N.A., Ibrahim, M., and Abbas, G. 2010. *In vitro* shoot and root proliferation of jack fruit as affected by different concentrations of growth regulators. *Sarhad J. Agric*. *26*(4): 533-538.

Ko, W.H., Su, C.C., Chen, C.L., and Chao, C.P. 2009. Control of lethal browning of tissue culture plantlets of Cavendish banana *cv*. Formosana with ascorbic acid. *Plant Cell TissueOrgan Cult.* 96(2): 137-141.

Kumar, R. and Tiwari, J.P. 2001. Effect of chemicals, light intensity and its duration on *in vitro* establishment of Chinese guava (*Psidium friedrichsthalianum*). *Prog. Hort*. 2001; 33(1): 1-6.

Kumar, V., Radha, A., and Chitta, S.K. 1998. *In vitro* plant regeneration of fig (*Ficus carica* L. cv. Gular) using apical buds from mature trees. *Plant Cell Rep.* 17(9): 717-720.

Lee, C.L. and Keng, C.L. 2005. Micropropagation of *Artocarpus heterophyllus* L. *Plant.* 81(956):689-695.

Li, B. and Han, G. H. 2003. Influence of nitrogen ratio and carbon sources on adventitious shoot regeneration of *Malus zumi* leaves *in vitro*. *J. Agric. Biotechnol*. 11(3): 253-258.

Litz, R.E., Moore, G.A., and Srinivasan, C. 1985. *In vitro* systems for propagation and improvement of tropical fruits and palms. *Hortic.Rev.* 7: 157-200.

Liu, X. and Yang, G. 2011.Clonal micropropagation of guava (*Psidium guajava* L.) on nodal explants of mature elite cultivar. *Int. J. Plant Biol.* 2(1): 7-10.

Mannan, M.A., Nasrin, H., and Islam, M.M. 2006. Effect of season and growth regulators on *in vitro* propagation of jackfruit (*Artocarpus heterophyllus* Lam). *Khulna Univ. Stud.* 7(2): 83-87.

Martin, K.P., Pachathundikandi, S. K., Zhang, C. L., Slater, A., and Madassery, J. 2006. RAPD analysis of a variant of banana (*Musa sp*.) cv. Grande Naine and its propagation via shoot tip culture. *In Vitro Cell. Dev. Biol. Plant*. 42(2): 188-192.

Meghwal, P.R., Singh, S.K., and Sharma, H. C. 2003. Micropropagation of aneuploid guava. *Indian J. Hortic.* 60 (1) : 29 – 33.

Melara, M.V. and Arias, A.M. 2009. Effect of BAP and NAA on shoot regeneration in cotyledonary explants of Costa Rican melon genotypes. *Agronomía Costarricense*, 33 (1): 125-131.

Miro, C.B. and Acedo, V.Z. 2013.Development of Micropropagation Protocol Supporting Sustainable Production of Jackfruit (*Artocarpus heterophyllus* Lam.). In: Acedo, A.L. and Kanlayanarat, S.(eds), *Proceedings of Second Southeast Asia Symposium on Quality Management in Postharvest Systems*, 2013, pp. 505-508

Murkute, M., Patil, S., and Singh, S.K. 2004. *In vitro* regeneration in pomegranate cv. Ganesh from mature plant. *Indian J. Hortic*. 61(3):206-208.

Nandwani, D. Purohit, S.D., Jain, S.M., and Ramawat .K.G. 2004. Propagation techniques in woody plants with special reference to arid and semi arid zone trees. In: Shanmugavel, P., Ignacimuthu, S. J (eds), *Tree improvement and biotechnology*. Pointer publishers, Jaipur, India, pp. 16-52.

Nimisha, C. 2016. Identification and characterization of jack fruit type (*Artocarpus heterophyllus*. Lam) in Kasargod district. MSc Hortic.Thesis, KAU, Thrissur.

Nishchal, N., Mir, H., Rani, R., and Pal, A.K. 2018.Effect of antioxidants in controlling phenol exudation in micropropagation of litchi cv. Purbi.*Curr. J. Appl. Sci. Technol*. 31(4): 1-7.

Nobre, J., Romano, A., Aksoy, U., Ferguson, L., and Hepaksoy, S. 1998. *In vitro* cloning of *Ficus carica* L. adult trees. *Acta Hortic.* 480: 161–164.

Nunjundaswamy, A.N. and Mahadevi, M. 1993. Fruit processing. In: Chadha, K.L. and Pareek, O.P. (eds). *Advances in Hort.4.* Malhotra Publishing House, New Delhi, pp. 1835-1875.

Park, S.Y., Kim, Y.W., Moon, H.K., Murthy, H.N., Choi, Y.H., and Cho, H.M. 2008. Micropropagation of *Salix pseudolasiogne* from nodal explants.*Plant Cell Tissue Organ Cult.* 93: 341–346

Pierik, R.L.M. 1987. *In vitro* culture of higher plants as a tool in the propagation of horticultural crops. *Acta Hortic.*226: 25-40.

Prabhuling, G., Rashmi, H., and Babu A.G. 2017.*In vitro* regeneration of jamun (*Syzygium cuminii* L.) cv. AJG-85.*Int. J. Sci. Nat*. 8 (4): 902 – 907.

Prakash, O., Kumar, R., Mishra, A., and Gupta, R. 2009. *Artocarpus heterophyllus* (Jackfruit): An overview. *Pharmacognosy Rev.* 3(6): 353–358.

Preece, J.E. 1995. Can nutrient salts partially substitute for plant growth regulators.*Plant Tissue Cult. Biotechnol.*1: 26-37.

Prem, J.V., Sajesh, N.K., Jiby, J.M., Ajesh, C.K., Benchamin, A., Renjith, J.M., Alen, N.A., Deenamol, T., Riny, S.T., Nijamol, V., and Sophyiamol, J. 2015. Chemistry and medicinal properties of jackfruit (*Artocarpus heterophyllus*): A review on current status of knowledge. *Int. J. Innovative Res. Rev* 3(2): 83-95.

Rahman, M.M., Amin, M.N., and Hossain, M.F. 2004. *In vitro* propagation of banyan tree (*Ficus benghalensis* L.)-A multipurpose and keystone species of Bangladesh. *Plant Tissue Cult.* 14(2): 135-142.

Ranasinghe, R.A.S.N., Maduwanthi, S.D.T., and Marapana, R.A.U.J. 2019. Nutritional and health benefits of jackfruit (*Artocarpus heterophyllus* Lam.): Rev. *Int. J. Food Sci.* 2019: 1-12.

Roh, S.K. and Hadiuzzaman, S. 1991. Micropropagation of two species of *Artocarpus* through *in vitro* culture. *Bangladesh J.Bot*.20: 27-32.

Roy, S.K. and Roy, P.K. 1996. *In vitro* propagation and establishment of a new cultivar of jackfruit (*Artocarpus heterophyllus*) bearing fruits twice yearly. In: Brumfield, R.G. (ed.), *Proceedings of Eighteenth International Symposium on Horticultural Economics*, 1996, pp. 497-502

Roy, S.K., Islam, M.S., Sen, J., Hossain, A.B.M.E., and Hadiuzzaman, S. 1993. Propagation of flood tolerant jackfruit (*Artocarpus heterophyllus*) by *in vitro* culture. *Proceedings of Second International Symposium on Horticultural Breeding*, 1992, pp. 273-278

Roy, S.K., Rahman, S.L., and Majumdar, R. 1990. *In vitro* propagation of jackfruit (*Artocarpus heterophyllus* Lam.). *J. Hortic Sci*. 65(3): 355-358.

Sáenz, L., Herrera-Herrera, G., Uicab-Ballote, F., Chan, J.L., and Oropeza, C. 2010. Influence of form of activated charcoal on embryogenic callus formation in coconut (*Cocos nucifera*). *Plant Cell Tissue Organ Cult.*100(3): 301-308.

Sajid, G.M., Ilyas, M.K. and Anwar, R. 2006. Effect of diverse hormonal regimes on *in vitro* growth of grape germplasm. *Pakist. J. Bot*. 38(2): 385-391.

Samaddar, H.N. 1990. Jackfruit. In: Bose, T.K. and Mitra, S.K. (eds), *Fruits: Tropical and Sub-tropical.* NayaProkash, Calcutta, pp. 638-649.

Scholten, H.J. and Pierik, R.L.M. 1998. Agar as a gelling agent: differential biological effects *in vitro. Sci. Hortic.* 77: 109-116.

Shahcheraghi, S.T. and Shekafandeh, A. 2016.Micropropagation of three endemic and endangered fig (*Ficus carica* L.) genotypes. *Adv. Hort. Sci.* 30 (3): 129–134.

Sharp, W.R., Evans, D.A., Ammirato, P.V., and Yamada, Y. 1984. *Hand Book of Plant Cell Culture.*Mac Publication Company, New York, pp 372-373.

Shatnawi, M., Shibli, R.A., Shahrour, W.G., Al-Qudah, T.S., and Taleb, A.Z. 2019. Micropropagation and Conservation of Fig (*Ficus carica* L.). *J. Adv. Agric*.10: 1669-1679.

Shatnawi, M.A., Johnson, K.A., and Torpy, F.R. 2004. *In vitro* propagation and cryostorage of *Syzygium francissi* (Myrtaceae) by the encapsulation-dehydration method. *In Vitro Cell. Dev. Biol.Plant*. 40(4):403-407.

Shibli, R.A., Shatnawi, M.A., Ajlouni, M.M., Jaradat, A., and Adham, Y. 1999. Slow growth *in vitro* conservation of bitter almond (*Amygdalus communis* L.). *Adv. Hortic. Sci.* 13: 133-134.

Shyamalamma, S., Chandra, S.B., Hedge, M., and Naryanswamy, N. 2008. Evaluation of genetic diversity in jackfruit (*Artocarpus heterophyllus* Lam.) based on amplified fragment length polymorphism markers. *Genet. Mol. Res. 7*(3): 645-656.

Singh, C.K., Raj, S.R., Jaiswal, P.S., Patil, V.R., Punwar, B.S., Chavda, J.C., and Subhash, N. 2016.Effect of plant growth regulators on *in vitro* plant regeneration of sandalwood (*Santalum album* L.) *via* organogenesis. *Agrofor. Syst.* 90(2): 281-288.

Singh, P. and Patel, R.M. 2014.Factors influencing *in vitro* growth and shoot multiplication of pomegranate. *Bioscan*. 9(3): 1031-1035.

Singh, S., Krishnamurthi, S., and Katyal, S.L. 1963.*Fruit Culture in India.* Indian Council of Agricultural Research, New Delhi, India. 451p.

Stuart, R. and Street, H.E. 1971. Studies on the growth in culture of plant cells. *J. Exp. Bot*. 22: 96-106.

Swami, S.B., Thakor, N.J., Haldankar, P.M., and Kalse, S.B. 2012. Jackfruit and its many functional components as related to human health: a review. *Comprehensive Rev. Food Sci. Food Saf*, 11(6): 565-576.

Torresm, C.K. 2013.*Tissue Culture Techniques for Horticultural Crops*. Van Nostrand Reinhold, New york, United States of America, 283p.

Tuia, V.S., Taylor, M.B., and Ragone, D. 2007. Studies on *in vitro* culture of breadfruit cultivars in the Pacific. *I Int. Symp. Breadfruit Res.. Dev. 757*: 161-168.

Vengadesan, G., Ganapathi, A., Amutha, S., and Selvaraj, N. 2002. *In vitro* propagation of *Acacia* species - a review. *Plant Sci.* 163(4): 663-671.

Vieitez, A.M. and Vieitez, M.L. 1980. Culture of chestnut shoots from buds in vitro. *J. Hortic. Sci.* 55(1): 83-84.

Wetherell, D.F. and Dougall, D.K. 1976. Sources of nitrogen supporting growth and embryogenesis in cultured wild carrot tissue. *Physiol. Plant*. 37: 97-

103.

Xiao, Z., Fu, R., Li, J., Fan, Z., and Yin, H. 2016. Overexpression of the Gibberellin 2-Oxidase gene from *Camellia lipoensis* induces dwarfism and smaller flowers in *Nicotiana tabacum. Plant Mol. Biol. Rep*. 34:182–91.

Zamir, R., Ali, N., Shah, S.T., Muhammad, T., and Shah, S.A. 2007. *In vitro* regeneration of guava (*Psidium guajava* L.) from shoot tips of mature trees. *Pakist. J. Bot*. 39(7): 2395-2398.

Abstract

DEVELOPMENT OF MICROPROPAGATION PROTOCOL FOR JACKFRUIT (*Artocarpus heterophyllus* Lam.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The present study entitled, "Development of micropropagation protocol for jackfruit (*Artocarpus heterophyllus* Lam.)" was conducted at tissue culture laboratory, Regional Agricultural Research Station, Pilicode, Kasaragod, during 2018-2020. All the experiments were laid out in completely randomized design. The first experiment was done with the objective to find out the best medium for culture establishment. The treatment combinations include T_1: MS medium with 2 mg/L BA, T_2: ½ MS medium with 2 mg/L BA, T_3: Modified with 2 mg/L BA, T_4: Woody plant medium with 2 mg/L BA, T_5: Gamborge B_5 medium with 2 mg/L BA replicated four times. The result revealed that T_1 took minimum days for shoot emergence (13.16), shoot multiplication & branching (15.83), produced maximum no. of shoots/ explant (1.83), Shoot length (1.13cm) and no. of leaves/ shoot (1.66).

Second experiment was done with the objective to find out effect of growth regulator for shoot multiplication with 5 treatments replicated four times. The treatment combinations included T_1: Selected basal medium, T_2: Best medium from the 1st experiment, T_3: T_2 + 1 mg/L BA, T_4: T_2 + 2 mg/L BA, T_5: T_2 + 3 mg/L BA. The study clearly indicated that T_3 showed better response in multiplication in terms of minimum days for shoot emergence (13), no. of shoots/ explant (1.99), no. of leaves/ shoot (1.66) and highest shoot length was noticed in T_2 (1.13). Another combination of growth regulators were also tried for multiplication with 8 treatments replicated thrice. The treatment combinations included T_1 : MS medium + 1mg/L BA + 0.35mg/L GA_3, T_2 : MS medium + 1.5mg/L BA + 0.35mg/L GA_3, T_3 : MS medium + 2mg/L BA + 0.35mg/L GA_3, T_4 : MS medium + 2.5mg/L BA + 0.35 mg/L GA_3, T_5 : MS medium + 3mg/L BA + 0.35mg/L GA_3, T_6 : MS medium + 3.5mg/L BA + 0.35mg/L GA_3, T_7 : MS medium + 4mg/L BA + 0.35mg/L GA_3, T_8 : MS medium + 4.5mg/L BA + 0.35mg/L GA_3. Results shows that MS medium + 2.5mg/L BA + 0.35 mg/L GA_3 found to be superior in terms of minimum days for shoot emergence (8.22 days), highest shoot length(2.26 cm), no. of leaves/ explant (4.44) and MS medium + 3mg/L BA + 0.35mg/L GA_3 produced maximum no. of shoots/ explant (3.88).

Third experiment was conducted with the objective to find out best growth regulator for rooting with nine treatments replicated thrice. Treatment combinations included T_1: ½ strength of selected basal medium, T_2: T_1 + 1 mg/L NAA, T_3: T_1 + 3 mg/L NAA, T_4: T_1 + 0.5 mg/L IBA, T_5: T_1 + 1 mg/L IBA, T_6: T_1 + 1 mg/L NAA + 0.5 mg/L IBA, T_7: T_1 + 1 mg/L NAA + 1 mg/L IBA, T_8: T_1 + 3 mg/L NAA + 0.5 mg/L IBA,T_9: T_1 + 3 mg/L NAA + 1 mg/L IBA. 0.4 g/L of activated charcoal was added to each treatment. None of the treatments produced roots. Another combination of growth regulators were also tried for rooting with eight treatments replicated thrice. The treatment combinations include T_1: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA, T_2: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA, T_3: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 0.5 mg/L IBA, T_4: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L IBA, T_5: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA + 0.5 mg/L IBA, T_6: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 1 mg/L NAA + 1 mg/L IBA, T_7: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA + 0.5 mg/L IBA, T_8: MS + 2.5 mg/L BA + 0.35 mg/L GA_3 + 3 mg/L NAA + 1 mg/L IBA. Rooting was not observed in all the treatment.

It can be concluded that among the basal medium, MS medium fortified with 2 mg/L BA is good for culture establishment. MS medium fortified with 2.5-3 mg/L BA along with 0.35 mg/L GA_3 can be used for shoot multiplication. Rooting was not observed because of basal callus formation and phenolic exudation.

സംക്ഷിപ്തം

പിലിക്കോട് പ്രാദേശിക കാർഷിക ഗവേഷണ കേന്ദ്രത്തിലെ ടിഷ്യു കൾച്ചർ ലബോറട്ടറിയിൽ 2018 -20 കാലയളവിൽ പ്ലാവിലെ തിരിയിൽ മൈക്രോപ്രൊപഗേഷൻ നടത്തുകയുണ്ടായി. ചിറപുറം ക്ലസ്റ്റർ ജാക്ക് (KJ 182) എന്ന പ്ലാവിനത്തിലെ തിരി ആണ് പഠനത്തിനായി ഉപയോഗിച്ചത്. ഷൂട്ട് വരുന്നതിനുള്ള മികച്ച മാധ്യമം, ഷൂട്ട് വിഭജനം ചെയ്യുന്നതിനുള്ള മികച്ച മാധ്യമം, വേര് വരുന്നതിനുള്ള മാധ്യമം കണ്ടെത്തുക എന്നിവ ലക്ഷ്യമിട്ടാണ് പഠനം നടത്തിയത്. ഷൂട്ട് വരുന്നതിനായി പ്രധാനമായും 5 മാധ്യമം ആണ് ഉപയോഗിച്ചത്. (എം എസ് മാധ്യമം, ഹാഫ് എം എസ് മാധ്യമം, മോഡിഫൈഡ് എം എസ് മാധ്യമം, ബി5 മാധ്യമം) എല്ലാ മാധ്യമത്തിന്റെ കൂടെയും 2 ppm ബി എ കൂടി ചേർക്കുകയുണ്ടായി. ഇപ്രകാരം കൾച്ചർ ചെയ്തതിൽ നിന്നും മനസിലാക്കാൻ കഴിഞ്ഞത് എം എസ് മാധ്യമത്തിന്റെ കൂടെ 2 ppm ബി എ കൊടുത്തത് മികച്ചരീതിയിൽ എസ്റ്റാബ്ലിഷ്മെന്റ് ചെയ്തു എന്നതാണ്. എം എസ് മാധ്യമത്തിൽ വെച്ച തിരികൾ എമർജൻസിനു വേണ്ടിയും (13 .16 ദിവസം) വിഭജനത്തിനായും (15.83 ദിവസം) കുറച്ചു ദിവസമാണെടുത്ത്, കൂടാതെ ഇതു ഏറ്റവും കൂടുതൽ ഷൂട്ടുകളും (1 .83) ഷൂട്ട് നീളവും ,ഇലകളും രേഖപ്പെടുത്തി.

ഒന്നാമത്തെ പരീക്ഷണത്തിൽനിന്നും മികച്ച മാധ്യമം ആയി തിരഞ്ഞെടുക്കപ്പെട്ട എം എസ് മാധ്യമമാണ് ഷൂട്ടിന്റെ വിഭജനത്തിനായി ഉപയോഗിച്ചത് . ഇതിൽ 1 ,1 .5 ,2 ,2 .5 ,3 ,3 .5 ,4 ,4 .5 mg/L BA യും, കൂടാതെ 0.35 mg/L GA_3 എന്ന ഹോർമോണും പഠനത്തിൽ ഉൾപ്പെടുത്തി . ഏറ്റവും കുറച്ചു ദിവസം ഷൂട്ട് വരുന്നതിനും (8 .2 2 ദിവസം), നീളം കൂടിയ ഷൂട്ട് (2 .2 6 cm), കൂടുതൽ ഇലകൾ/ ഷൂട്ട് എന്നിവ വരുന്നതിനും 2.5 mg/L GA_3 ഉള്ള എം എസ് മാധ്യമമാണ് മികച്ചതെന്നും കണ്ടെത്തുകയുണ്ടായി. കൂടാതെ ഏറ്റവും കൂടുതൽ ഷൂട്ട് ഉത്പാദനം നടന്നത് 3 mg/L BA, 0.35 mg/L GA_3 ഉള്ള മാധ്യമത്തിൽ ആണെന്നും

മനസിലാക്കാൻ കഴിഞ്ഞു .

വേര് വരുന്നതിനായി ഹാഫ് എം എസ് മാധ്യമം മാത്രവും (കേവല നിയന്ത്രണം), ഇതിന്റെ കൂടെ NAA (1, 3 mg/L), BA (0.5, 1 mg/L) എന്നിവയും ഉപയോഗിച്ചു. എന്നാൽ ഒരു മാധ്യമത്തിലും വേര് വന്നതായി കാണപെട്ടില്ല .

Annexure

Annexure 1

Table 1: MS medium composition and stock preparation

	Ingredients	Quantity mg/ L	Concentration of stock	Quantity required g/ L	Volume of stock	Vol. req. for 1L media
Macro elements Stock A	$NH_4 NO_3$	1650	10 X	16.5	500 ml	100 ml
	KNO_3	1900		19		
	$MgSO_4.7H_2O$	370		3.7		
	$KH_2 PO_4$	170		1.7		
	CaCl2. 2H2O	440		4.4		
Micro elements Stock B	KI	0.83	100 X	0.083	500 ml	10 ml
	H_3BO_3	6.20		0.62		
	$MnSO_4. 4H_2O$	22.3		2.23		
	$ZnSO_4. 4H_2O$	8.6		0.860		
	$Na_2MoO_4 .2H_2O$	0.25		0.025		
	$CuSO_4. 5H_2O$	0.025		0.0025		
	CoCl2. 6H2O	0.025		0.0025		
Iron source Stock C	$FeSO_4. 7H_20$	27.85	100 X	2.785	500 ml	10 ml
	Na_2 - EDTA	37.25		3.725		

Vitamins Stock D	Thiamine HCl	0.1	100 x	0.01	100 ml	10 ml
	Niacin	0.5		0.05		
	Glycin	2.0		0.2		
	Pyridoxine HCl	0.5		0.05		
	Myoinositol	100				
Carbon source	Sucrose	30g/L				
	Agar	6g/L				

Table 2: Half MS medium composition and stock preparation

	Ingredients	Quantity mg/ L	Concentration of stock	Quantity required g/ L	Volume of stock	Vol. req. for 1L media
Macro elements Stock A	$NH_4 NO_3$	1650	10 X	16.5	500 ml	50 ml
	KNO_3	1900		19		
	$MgSO_4.7H_2O$	370		3.7		
	$KH_2 PO_4$	170		1.7		
	$CaCl_2. 2H_2O$	440		4.4		
Micro elements Stock B	KI	0.83	100 X	0.083	500 ml	5 ml
	H_3BO_3	6.20		0.62		
	$MnSO_4. 4H_2O$	22.3		2.23		
	$ZnSO_4. 4H_2O$	8.6		0.860		
	$Na_2MoO_4 .2H_2O$	0.25		0.025		
	$CuSO_4. 5H_2O$	0.025		0.0025		
	CoCl2. 6H2O	0.025		0.0025		
Iron source Stock C	$FeSO_4. 7H_20$	27.85	100 X	2.785	500 ml	5 ml
	Na_2 - EDTA	37.25		3.725		

Vitamins Stock D	Thiamine HCl	0.1	100 x	0.01	100 ml	5 ml
	Niacin	0.5		0.05		
	Glycin	2.0		0.2		
	Pyridoxine HCl	0.5		0.05		
	Myoinositol	50				
Carbon source	Sucrose	15g/L				
	Agar	3g/L				

Table 3: Modified MS medium composition and stock preparation

	Ingredients	Quantity mg/ L	Concentration of stock	Quantity requiredg / L	Volume of stock	Vol. req. for 1L media
Macro elements Stock A	$NH_4 NO_3$	1650	10 X	16.5	500 ml	50 ml
	KNO_3	1900		19		
	$MgSO_4.7H_2O$	370		3.7		
	$KH_2 PO_4$	170		1.7		
	CaCl2. 2H2O	440		4.4		
Micro elements Stock B	KI	0.83	100 X	0.083	500 ml	10 ml
	H_3BO_3	6.20		0.62		
	$MnSO_4. 4H_2O$	22.3		2.23		
	$ZnSO_4. 4H_2O$	8.6		0.860		
	$Na_2MoO_4 .2H_2O$	0.25		0.025		
	$CuSO_4. 5H_2O$	0.025		0.0025		
	CoCl2. 6H2O	0.025		0.0025		
Iron source Stock C	$FeSO_4. 7H_20$	27.85	100 X	2.785	500 ml	10 ml
	Na_2 - EDTA	37.25		3.725		

Vitamins Stock D	Thiamine HCl	0.1	100 x	0.01	100 ml	10 ml
	Niacin	0.5		0.05		
	Glycin	2.0		0.2		
	Pyridoxine HCl	0.5		0.05		
	Myoinositol	100				
Carbon source	Sucrose	30g/L				
	Agar	6g/L				

Table 4: Woody plant medium composition and stock preparation

	Ingredients	Quantity mg/ L	Concentration of stock	Quantity requiredg / L	Volume of stock	Vol. req. for 1L media
Macro elements Stock A	$NH_4 NO_3$	400	10 X	4	500 ml	100 ml
	$CaCl_2$. $2H_2O$	72.5		0.725		
	$CaNO_3.H_2O$	386.34		3.86		
	$MgSO_4.7H_2O$	180.69		1.80		
	$KH_2 PO_4$	170		17		
	K_2SO_4	990		9.9		
Micro elements Stock B	H_3BO_3	6.20	100 X	0.62	500 ml	
	$CuSO_4$. $5H_2O$	0.025		0.0025		
	$MnSO_4 .H_2O$	22.3		2.2		
	$ZnSO_4$. $7H_2O$	8.6		0.86		
	Na_2MoO_4 $.2H_2O$	0.213		0.0213		
Iron source Stock C	Na_2 - EDTA	37.300	100 X	3.73	500 ml	10 ml
	$FeSO_4$. $7H_20$	27.500		2.75		

Vitamins Stock D	Thiamine HCl	1	100 x	0.1	100 ml	10 ml
	Nicotinic acid	0.5		0.05		
	Glycin	2.0		0.2		
	Pyridoxine HCl	0.5		0.05		
	Myoinositol	100				
Carbon source	Sucrose	30g/L				
	Agar	6g/L				

Table 5: B5 medium composition and stock preparation

	Ingredients	Quantity mg/ L	Concentration of stock	Quantity requiredg / L	Volume of stock	Vol. req. for 1L media
Macro elements Stock A	$NH_4 SO_4$	134	10 X	1.34	500 ml	100 ml
	KNO_3	2500		25		
	$MgSO_4.7H_2O$	122.09		11.22		
	$Na_3 PO_4$	130.42		1.30		
	CaCl2. 2H2O	113.230		1.13		
Micro elements Stock B	H_3BO_3	3	100 X	0.3	500 ml	10 ml
	CoCl2. 6H2O	0.025		0.0025		
	$MnSO_4. 4H_2O$	10		1		
	$ZnSO_4. 6H_2O$	2		0.2		
	$Na_2MoO_4 .2H_2O$	0.213		0.0213		
	$CuSO_4. 5H_2O$	0.025		0.0025		
	KI	0.750		0.075		
Iron source Stock C	$FeSO_4. 7H_20$	27.85	100 X	2.785	500 ml	10 ml
	Na_2 - EDTA	37.25		3.725		

Vitamins Stock D	Thiamine HCl	10	100 X	1	100 ml	10 ml
	Pyridoxin HCl	1		0.1		
	Nicotinic acid	1		0.1		
	Myoinositol	100				
Carbon source	Sucrose	30g/L				
	Agar	6g/L				